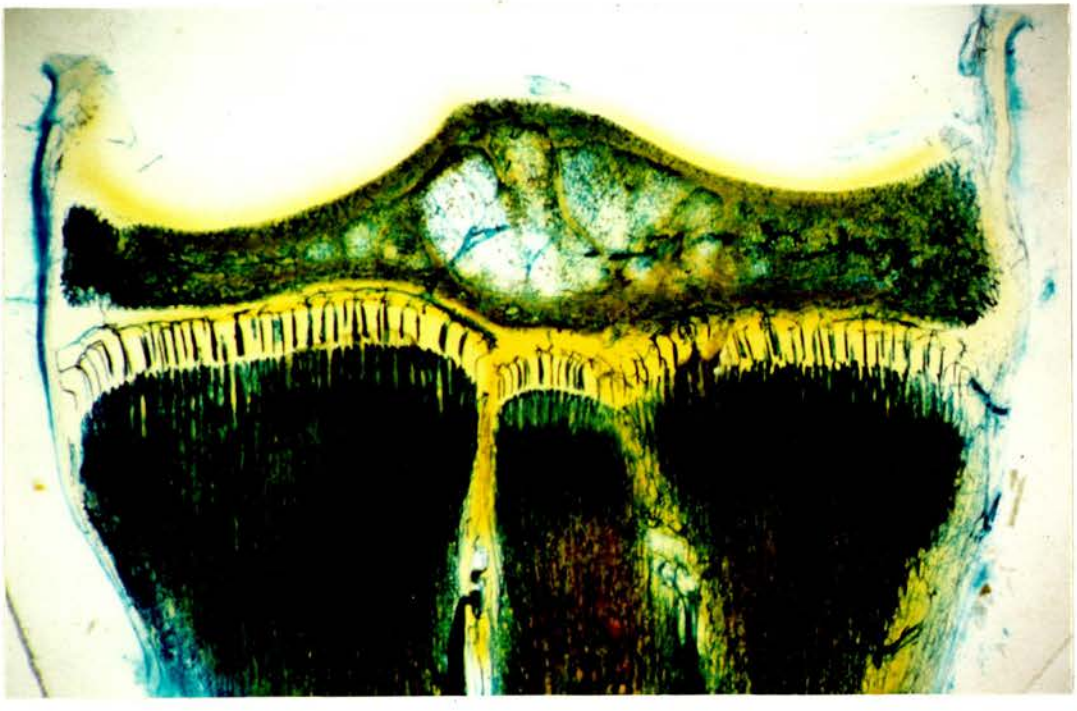
THE EFFECT OF GENOTYPE AND ENVIRONMENT ON GROWTH AND VASCULARITY OF THE PELVIC APPENDICULAR SKELETON IN THE FOWL

BARRY. H. THORP.



A thesis submitted for the Degree of Doctor of Philosophy.

University of Edinburgh. May 1986.

DECLARATION

I declare that this thesis has been composed by me, and that the work is my own.

3rd May 1986.

ABSTRACT

A method is described which enables visualization of the blood supply in developing long bones. The same material was also suitable for the preparation of undecalcified histological sections. The circulatory system was perfused with a solution of dye and barium sulphate. The skeletal tissue was cleared in plastic resin before embedding and tissue blocks were cut into 1mm slabs.

Laying strain and broiler fowls were reared from hatching till twenty weeks of age. Birds were killed throughout the growth period and specimens prepared for study. Gross morphological features were recorded. The origin and nature of cartilage canals were established in the bone extremities of the proximal and distal femur, proximal and distal tibiotarsus and proximal tarsometatarsus. Similar studies were performed on small groups of broiler fowls reared under different environmental conditions. Vascularity of bone extremities and morphological features were compared between the groups of fowls. Growth rate in normal and abnormal bone extremities was investigated. The involvement of vascularity in the formation and repair of dyschondroplasia was elucidated.

The fundamental vascular patterns were similar in different genotypes of fowl, and similarities were apparent with vascular patterns reported in other species. Disrupted endochondral ossification in conjunction with vascular abnormalities occurred in every group of fowls. In ad libitum fed broiler fowls, lesions

were most extensive, and contributed to limb asymmetry and a wide range of bone torsion. Enviromental factors greatly affected the number, range and extent of abnormalities. In some broiler fowls, the rate of lesion formation appeared to exceed that of repair. The behavioural characteristics of broilers under certain environmental conditions was detrimental to the maintainance of adequate vascular perfusion in bone extremities.

Table of Contents

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to the director, Dr D.W.F.Shannon, and staff of the Poultry Research Centre for assistance and the provision of facilities.

The late Professor J.R.Campbell gave enthusiastic supervision during the initial stages of this study. The role of supervisor was undertaken by Mr.J.A.Fraser, whose ungrudging support was invaluable in the completion of this work.

This study could not have been performed without the assistance of considerable technical expertise in the Photographic and Metabolic Pathology departments of the Poultry Research Centre. Special mention must be made of the contribution by Mr.R.K.Field for the quality of photographic reproduction in this thesis.

Throughout, Dr.S.R.I.Duff has been a source of inspiration, advice and invaluable guidance. Finally, my thanks to my parents for their encouragment during this period of further academic studies.

INTRODUCTION

Genotype and environment are both important in the development of normal and abnormal skeletal morphology. The present study set out to elucidate the influence of these factors, and to establish their individual importance, on pelvic limb development in the fowl.

Particular emphasis has been placed on vascular studies as abnormalities in blood supply are considered to be of paramount importance in the development of certain orthopaedic conditions.

The initial stages of this investigation aimed to establish the patterns of skeletal morphology and vascularity in the pelvic appendicular skeleton of the fowl. In order to examine the vascularity of individual long bones in the fowl, perfusion and clearing methods had to be developed. If possible, it was hoped that such methods would permit the same tissues to be subsequently available for histological investigation.

Studies were performed on a slow growing strain of fowls and fast growing broiler fowls. In such a manner the range of normality within each genotype of fowl was established. In addition to this genotypic effect, certain environmental factors were investigated. In particular the effects of exercise and feed restriction were studied in broiler fowls.

Some aspects of this study were investigated in greater depth. These further investigations assessed the effect of localized lesions on bone growth. the relationship between growth

rate and physeal (growth plate) thickness and the formation and repair of developmental lesions in bone extremities.

The fundamental nature of the present investigation makes it of value in the study of developmental orthopaedic disease in other species.

LIST OF ABBREVIATIONS

Ac = accessory ossification centre
CA = caudal
CR = cranial
D = dyschondroplastic defect
dis = distal
E = epiphysis
ECRV = extra-capsular retinacular vessel
EOC = epiphyseal ossification centre
EVC = epiphyseal vascular canal
Fib = fibula
FH = femoral head
FT = femoral trochanter
GP = growth plate / physis
Hy = hypotarsus
ICRV = intra-capsular retinacular vessel
IAV = intra-articular vessel
JC = joint capsule
L = lateral
M = medial
MGT = Mason Goldner trichrome
Met = metaphysis
MV = metaphyseal vessel
P = physis
PEV = penetrating epiphyseal vessel
PR = perichondrial ring
prox = proximal
Tib = tibiotarsus
TLV = teres ligament vessel
TM = tarsometatarsus
TT = tibiotarsus
UWB = unilateral weight bearing
VF = vascular foramen
II, III and IV = 2nd, 3rd and 4th metatarsals
3rd = third EOC in distal tibiotarsus

A copy of this list is inserted into a pocket in the front cover.

EXPERIMENT 1:Perfusion.

INTRODUCTION

The use of perfusion techniques in the study of skeletal vascularity is not new. Hunter (1742, cited by Harris, 1929) as a result of a series of injection studies of the limb identified the "circulus vasculosis articuli". This circulus vasculosis articuli was not only the vascular supply to the joint and intra-articular structures, but also the supply to the epiphysis in relation to the joint capsule (Harris. 1929).

In the study of tissue vascularity early workers used injection methods to prepare vascular casts of the vessels. These injection methods did not allow the visualisation of intraosseous vasculature. The formation of casts only provided information on the origin and point of entry of vessels into the skeleton. However with the technique of vascular casts Langer (1876) recognised cartilage canals in the relatively translucent cartilage of the foetal skeleton. The vascular cast technique with modern resin preparations is the method of choice in demonstrating the extraosseous vascular supply and produces excellent results (Kaderly et al, 1982).

No further advances were made in the investigation of intraosseous vascularity until radiology became available. The microangiographic examination of perfused tissue by the preparation of radiographs is referred to as microradiography.

One of the great pioneers at the start of this century was Lexer (1904, cited by Tilling, 1958). He injected a contrast media of mercury and turpentine and on completion of injection, stereoscopic radiographs of the skeletal tissue were produced. Sixty years later this technique was still being used by Heraldsson (1962). who perfused specimens with "Plumbi subcubonis. Sol natri clor physiolog and Gummi arabicum" as a radiopaque medium. Heraldsson (1962) also used stereoscopic radiographs to form a three dimensional image of the perfused tissue. A mixture of starch and lead was the perfusate prepared by Rodgers and Gladstone (1950).

Perfusion methods were not standardised with researchers using a variety of different substances in their perfusion studies. The situation improved when barium sulphate, in a purified form and uniform particle size (Micropaque) became available. From the 1950's micropaque was widely used in the perfusion mixtures for microradiography (Trueta and Harrison, 1953; Okawa and Trombra, 1956; Peterson et al, 1957; Brookes, 1958a; Tilling, 1958; Rhindlander and Baraying, 1962 and Crock, 1967). The size of the barium sulphate particles in micropaque results in filling of the arterial circulation but the particles do not easily pass into the venous circulation (Ardan, 1953).

The majority of present day investigations by microradiography of skeletal vascularity still rely on barium sulphate as the radiodense material in the perfusion mixture. Recent studies of the physeal vascularity in young foals (Firth and Poulos, 1983) and in young pigs (Hill et al,1985) used barium

sulphate in the perfusion mixture prior to microradiography.

There have been few investigations into the relationship between the information obtained by microradiography and the actual intravital microscopic anatomy. Lundskog et al (1968) compared the intravital and microradiographic anatomy of rabbit mesentery and of hamster's cheek pouch with respect to the degree of filling. The perfusate contained barium sulphate in the form of micropaque. They concluded that it was possible to visualise vessels of 50u to 100u. , but a complete identification of all the capillaries was not achieved by standard microradiographic techniques. There was also less distortion in the architectural structure in denser tissue such as a rabbit ear compared to a relatively loose tissue such as cheek pouch or mesentery. This suggests that in the experimental work of this thesis, which is a study of the skeleton, there should be little structural distortion of the vessels due to the rigidity of the tissue.

Even when vessels are represented they cannot be considered to be true to life. Suoranta and Kormano (1974) in their detailed study of the morphology of rabbits ears represented by microradiography and the actual intravital anatomy, noted changes in the delineation of blood vessels including local narrowing, breakages of contrast pillars, uneven distribution of contrast materials and hollows in the contrast pillars.

The reproduction of the vascular pattern in perfused skeletal tissue by microradiography sometimes makes the differentiation between artefact and vascular abnormality difficult. The examination of perfused tissue was much advanced by the advent of

clearing methods. Clearing caused the skeleton to become transparent and allowed the direct viewing of perfused vessels and aided in the identification of artefact. Prior to clearing, the blood vessels would have been perfused with a coloured dye. In the cleared specimen any vessel that had been stained by the dye or contained dye would be visible. The preparation and examination of the vasculature in tissues which have been perfused and subsequently cleared is referred to microangiography.

Spalteholz (1914) first described the clearing of skeletal tissue with a mixture of methyl salicylate and benzyl benzoate. This mixture has been used in various modified forms by many workers including Trueta and Morgan (1960),Bassett et al (1967) and Crock (1967).

Even when clearing methods are used barium sulphate is frequently included in the perfusion mixture. It was found that dyes such as Berlin blue adhered to the barium sulphate and were not removed by subsequent processing or histological processing or clearing in Spalteholz fluid. The particles of barium sulphate maintain both the shape of the vessels and the presence of the dye in the vessel (Sevitt 1964).

Various substances have been injected into the circulation prior to perfusion. The injection of heparin into experimental animals prevents the blood clotting and occluding vessels. This technique cannot be applied to clinical material. Of course in studies of skeletal vascularity in man, such as those by Trueta and Harrison (1953), it was not possible to inject anticoagulants.

Vasodilators are utilised to maximise vascular perfusion.

Hexamethonium injected prior to perfusion was used by Okawa and Trombka (1956) as a vasodilator. A 2% solution of lignocaine was injected intravenously prior to perfusion by Hill et al (1985). Vasodilators can also be incorporated into the perfusion mixture, such as sodium nitrate (Kelly et al, 1968), lignocaine (Lundskog et al, 1968) and sodium citrate (Rubin, 1964).

There is great variation in the types of perfusion apparatus that have been developed. Techniques include the use of a syringe and manual force (Trueta and Buhr, 1963; Beaumont, 1967). , with no means of measuring perfusion pressure. Alternatively methods using moniters and a pulsators have been used, which resembles the systolic and diastolic pressures in the live animal.

Many different pressures have been used in the perfusion of skeletal tissues (Table A). The majority of pressures selected, however, do fall into the range of normal systolic to diastolic blood pressure. The ideal pressure would be adequate to ensure filling of all functional vessels in all specimens and yet not cause damage to the vascular architecture.

There has also been variation in the methods of estimating the conclusion of perfusion in an individual specimen. Sevitt (1964), when perfusing with Berlin blue to prepare specimens of the femoral head, considered that successful injection of perfusate was heralded by blueing of the skin over the upper thigh and the return of blue fluid from the internal iliac artery. Brookes and Landon (1964), in their study of the skeletal vascularity in the foetus with indian ink, considered that perfusion was complete when the skin was deeply and completely

---

TABLE A

The perfusion pressures selected by different investigators of skeletal vascularity

PRESSURE	SPECIES	INVESTIGATOR
200mm Hg	rabbit	Okawa and Trombka (1956).
150mm Hg	rabbit	Gothman (1960).
120mm Hg	dog	Rhinelander (1962).
150mm Hg	rat	Rubin (1964).
300mm Hg	adult man	Crock (1967).
200mm Hg	child	Crock (1967).
110mm Hg	dog	Bassett et al (1969).
130mm Hg	rabbit	Albrektsson (1981).
110mm Hg	cat	Pohlymeyer (1981).
120mm Hg	dog	Kaderly et al (1983).

---

black. In other studies, where entire specimens were being perfused prior to dissection of the skeleton, Brookes (1958a and 1958b) continued perfusion until the vessels of the intestinal wall were full of perfusate and all an intense black. The methods of some workers centred around the injection of a premeasured quantity of perfusate (Trueta and Buhr, 1963; Albrektsson, 1981); whilst other workers maintained perfusion pressure for a fixed period of time (Lundskog, 1968; Hansen-Letch, 1979). In other studies perfusion pressure was maintained until perfusate flowed

from the venous system (Okawa and Trombka, 1956; Kaderly et al, 1982 and Hill et al, 1985).

The purpose of this experiment was to establish a technique of vascular perfusion in the skeleton of the fowl which would produce consistent results.

MATERIAL AND METHODS.

In this study a multipurpose perfusion apparatus (Rothwell et al.1973) was used (Fig 1). This apparatus was designed to provide a source of perfusate at a predetermined pressure and at a controlled rate of flow. Measurements of pressure were provided by an anaeroid sphygmomanometer. The apparatus was modified by the addition of a magnetic stirrer to the round bottomed flask of perfusate.

The perfusion mixture was made up of 17.5% barium sulphate (Micropaque powder), 3.5% sodium citrate and 2% Berlin blue in a 10% solution of buffered neutral formalin.

All specimens to be perfused were injected intravenously with heparin, prior to being killed by barbiturate overdose. The thorax was then opened and the aorta catheterised. In larger birds an incision was made in the wall of the aorta and a catheter was inserted in the direction of blood flow. In the younger birds the catheter was inserted via an incision into the left ventricle and from there into the aorta. A ligature was then placed around the aorta to retain the catheter in position and prevent leakage of perfusate. The catheter could then be connected to the perfusion apparatus and the pelvic limbs perfused. The pelvis and attached limbs were then dissected from the bird. Twelve birds of two weeks of age were perfused at different pressures. Two birds were each perfused at 80, 100, 120, 140, 150 and 160mm Hg. At each pressure one bird was perfused for five minutes and the second for ten.

Fig 1. The multipurpose perfusion apparatus.

Fig 2. Three tibiotarsi perfused at 100mm Hg, 120mm Hg and 140mm Hg respectively. The intensity of the blue colour in the skeleton is greatest at 140mm Hg where the dye can be observed in the cartilage of the bone extremities.

The efficacy of perfusion was judged by the intensity of the blue colouration in the skeleton of the pelvic appendicular skeleton.

The technique of preparing 1mm slabs of perfused tissue is explained in experiment 2. However the appearence of perfused vessels is relevant to this section and the method of perfusion. The appearance of the perfusate filled vascular canals is therefore included in these results.

RESULTS

The perfusion pump was easy to use and produced consistent results. The addition of the magnetic stirrer maintained a suspension of barium sulphate in the perfusate.

The efficacy of perfusion was judged on a scale of 0 to 8/8. 0 was where there was no blue colouration of the skeleton and 8/8 was where the skeletal tissue was coloured an intense blue throughout (Fig 2 and Table B). The limbs perfused at 120mm Hg or less took longer to colour and the skeleton was either a very pale blue or there was uneven distribution of the dye. In the specimens perfused at a pressure greater than 150mm Hg there was rupture of some of the subcutaneous blood vessels which allowed leakage of the dye into the surrounding tissues. All the skeletal tissue in this trial perfused at a pressure greater than 140mm Hg were an intense blue throughout. It was noted that in all the specimens that were satisfactorily perfused the skin overlying the area of interest had become an intense blue and blue dye was leaking from vessels unavoidably severed during the dissection to catheterise the aorta.

The slabs were examined with a binocular microscope. A camera was attached to a side arm of the microscope. Many of the slabs were photographed, the majority in black and white. A few colour photographs were also prepared. These colour photographss closely resemble the image of the slabs when viewed through the binocular microscope (frontpiece).

Examination of cleared slabs from the bone extremities of

TABLE B

The effect of different pressures and times on the efficacy of perfusion.

Preasure (mmHg).	Time (minutes).	Efficacy of Perfusion Right.	Left.	Vessel Rupture.
0	5	4/8	0	-
80	10	3/8	5/8	-
100	5	6/8	4/8	-
100	10	6/8	5/8	-
120	5	6/8	6/8	-
120	10	5/8	4/8	-
140	5	4/8	5/8	-
140	10	5/8	8/8	-
150	5	8/8	8/8	-
150	10	7/8	8/8	-
160	5	8/8	8/8	+
160	10	8/8	8/8	+

---

perfused specimens demonstrated even and consistent filling of the vascular canals.

Under higher magnification barium sulphate was apparent in the arterial system. The larger veins did not contain barium sulphate but the lumen was stained blue by the Berlin blue. The

arterial and venous components of the smaller vessels in the bone extremities were not discernible.

DISCUSSION.

The mixture of perfusate was a modification of the mixture used by Rietz (1968) in his experimental work in the dog. The mixture was altered by the addition of Berlin blue and replacing the saline with 10% buffered neutral formalin. The addition of a dye was because the tissues were to be cleared, whilst Reitz relied on microradiography to record the vascular pattern. The purpose of substituting buffered neutral formalin for saline was to fix the tissues as early as possible during processing , reducing the effect of post mortem changes on subsequent histology.

The use of the modified perfusion apparatus produced consistent results without the problems reported by Hill et al (1984) From this trial a pressure between 140 and 150mm Hg was selected for perfusion in the future experiments. In all subsequent experiments the perfusion pressure was maintained only until the skin overlying the area of interest had become an intense blue, and also blue dye was leaking from the vessels that had been unavoidably severed during the surgical approach to the aorta. This technique of preparing perfused specimens was followed in all the experimental work performed in these studies.

A total of 310 birds were perfused. Only the occasional specimen was poorly perfused. There were no specimens with rupture of vessels, due to an excessive perfusion pressure. Occasionally epiphyseal vascular canals did not contain any perfusate, although the gross specimen had apparently been

satisfactorily perfused. These "ghost" vessels were then studied histologically to ascertain whether they were patent vascular canals or vessels that were occluded in vivo. Infrequent presence of patent "ghost" vessels demonstrated the efficacy of the perfusion technique.

Rubin (1964) described how to achieve a high degree of versimilation in perfusion studies.

" The variables of the method must be appreciated, and exacting standards and conditions of injection employed. Otherwise fact is replaced by artefact; and qualitative and quantitative evaluations become impossible."

The main object is to keep the conditions of injection as physiological as possible.

The success of perfusion in the present study was due to a recognition of the importance of Rubin's guiding principals of perfusion. Some system is advised to control the level of pressure, as it is impossible to maintain a constant pressure by hand injection. In a dead animal it is impossible to maintain physiological conditions during perfusion. Ideally perfusion should be performed when the effects of post mortem change are minimal. The method of perfusion should take into account the physiological state of the tissues.

There are two major causes of artefacts. Underfilling which suggests avascular areas. Overfilling which causes vessel rupture and resembles haemorrhage.

There are a number of points which contributed to the quality of the perfused specimens.

All the material perfused had been injected with heparin and was freshly killed. This reduced the probability of blood coagulation interfering with the flow of perfusate.

In each canal there are a number of vessels, including arteries, veins and capillaries, and only one of these vessels requires to contain perfusate for that canal to be demonstrated.

A relatively high perfusion pressure was used compared to that of many other workers. The technique employed ensured good filling of vessels, but there was no overfilling or vessel rupture as the pressure was carefully controlled and in the normal physiological range.

There was no sectioning of the perfused tissues prior to embedding, therefore there was no leakage or loss of perfusate from the canals.

EXPERIMENT 2:The clearing and examination of perfused skeletal tissue

INTRODUCTION

Microradiography is a method which has been frequently used in the examination of skeletal vascularity. It is not necessary to decalcify specimens, which can be prepared for histological examination after the microradiographs have been prepared. This technique is suitably demonstrated by Rhinelander in his studies (Rhinelander and Baragray, 1962; Rhinelander, 1968; Rhinelander et al, 1968). There are, however, disadvantages to microradiography. The specimen has to be cut into slabs before microradiography, when perfusate can be lost during slicing of the tissue. If vessels are not full of perfusate they will not be delineated on the microradiograph. The "ghost" vessels which can occur in cleared specimens are not apparent on a micoradiograph. The examination of apparent or true defects in vascular filling requires the examination of many serial sections. To ensure that the correct area is examined the microradiographed specimen must be prepared and cut with precision.

Sevitt (1964) considered that, in the study of the vasculature in perfused skeletal tissue, the examination by microangiography of cleared specimens was superior to microradiography

Spalteholz's technique has been widely used in the production

of cleared specimens of skeletal tissue. Skeletal tissue which has been cleared by the Spalteholz method can be further processed for histological examination. Material which is prepared by the Spalteholz technique is often distorted during processing, with slabs undulating across their surface. This makes further processing and the production of representative histological sections impossible.

Prior to clearing in Spalteholz fluid the bone is decalcified (Sevitt, 1964; Beaumont, 1967). The decalcification of Spalteholz cleared material is detrimental to future histological examination. Bonucci and Reurink (1978) in their study of skeletal ultrastructure compared the effect of decalcification on specimens prior to and after embedding in resin. They concluded that when decalcificaton preceded embedding, it invariably removed all the inorganic substance, but at the same time, and probably as a result of the solubilisaton of organic substance, the fine structure of cells was so drastically changed by this treatment that it was sometimes impossible to recognise any cell structure. When decalcification was performed after embedding then the resin considerably reduced or completely prevented the solubilisation of organic material.

To circumvent the loss of information, due to decalcification, thick and thin slabs of tissue can be prepared from the perfused specimen being studied (Trueta and Harrison, 1953; Crock, 1967 and Duff, 1979). The thick slabs were examined after clearing in Spalteholz solution. The thin slabs were then processed for histological examination. This method, however,

made the accurate description of histology relative to the actual local vascularity difficult. The vascular supply to tissue can change markedly over a very short distance.

Plastics have become popular as embedding media for tissue because of the advantages they offer over softer media. Tissue shrinkage is minimal, being about 2% as compared with between 10% and 50% in wax (Velde et al, 1977). The hard nature of some plastics means that they are ideal for the preparation of sections of undecalcified bone.

One of the first plastic resins to be used to produce undecalcified bone sections was methyl methacrylate (Newman et al, 1949). In a study of the normal arterial pattern in rabbit cortical bone Gothman (1960) used methyl methacrylate to embed specimens for both stereomicroangiographic and histological study. The method of preparing tissue blocks of methyl methacrylate can be dangerous and has been modified in an attempt to make the procedure safer (Difford, 1974). There are however still problems with the size of specimens that can be processed in methyl methacrylate.

Polymaster 1209AC has been found to be the most satisfactory plastic resin for producing undecalcified bone sections from large necropsy and surgical specimens of bone (Mawhinny and Ellis. 1983). Polymaster resin is a plastic resin incorporating a styrene monomer stabilised by hydroquinone. Addition of an organic peroxide "catalyst" polymerises the resin by addition reactions between unsaturated C=C bonds in the plastic monomer, crosslinked with the C=C bonds in the styrene. The resulting

polymer is extremely inert, forming a hard insoluble plastic that tolerates most staining reactions commonly used in histology laboratories. In addition it permits more specialised bone staining techniques such as von Kossa, Toluidine blue, Masson Goldner trichome and Movats pentachrome. The resin is manufactured in large quantity and is of low cost (Mawhinny and Ellis. 1983).

Hibben (1937) prepared museum specimens in methyl methacrylate because of its transparent nature. Reinhold et al (1983) reports the use of epoxy resin to produce clear blocks of embedded tissue, which they describe as similar to specimens cleared in Spalteholz fluid.

The purpose of this experiment was to establish a method for clearing skeletal tissue to enable the accurate examination of perfused vascular canals. In addition it was required that this same tissue was made available for undecalcified histological study after the vascular pattern of the specimen had been examined and recorded.

Some plastic resins appeared to have the ability to clear tissues and others to be suitable for the production of undecalcified bone sections. A trial was carried out to assess the suitability of Polymaster resin in the preparation of perfused skeletal tissue for both vascular and histological studies.

MATERIAL AND METHODS.

The perfused limbs from the previous experiment were used in the first Polymaster trial. The three principal pelvic long bones were dissected from each limb. The proximal and distal bone extremities from the femur and tibiotarsus and the proximal tarsometatarsus were then fixed in 10% buffered neutral formalin.

The bone extremities were infiltrated with and embedded in Polymaster resin (Bondaglass-Voss Ltd) using the technique developed by Mawhinny and Ellis (1983). This method centres around the use of cellosolve (2-ethoxyethanol) to dehydrate specimens prior to infiltration with resin.

The processing of the bone extremities was modified in future experiments. Alcohol was used to dehydrate specimens instead of cellosolve. After dehydration in alcohol the specimens were transfered to pure Polymaster resin. The specimen remained in the Polymaster until it had cleared. The resin infiltrated blocks were positioned for embedding in plastic disposable moulds. The moulds containing the polymerising resin were placed in a shallow water bath. The bath acted as a heat sink dissipating the heat from the exothermic reaction and preventing rapid polymerisation. Too rapid a polymerisation could result in cracks in the plastic blocks. The resin blocks were trimmed with a bandsaw to remove excess plastic. Individual bone extremities were then mounted in tan wax on a glass slide (Fig 4), (Duff, 1979). A precision annular saw (Microslice 2, Metals Research) was used to slice the blocks into 1mm thick slabs (Fig 3). The blade was of a 240

Fig 3. A resin embedded specimen held firmly by tan wax to a glass slide which in turn is bonded by tan wax to the saw table.

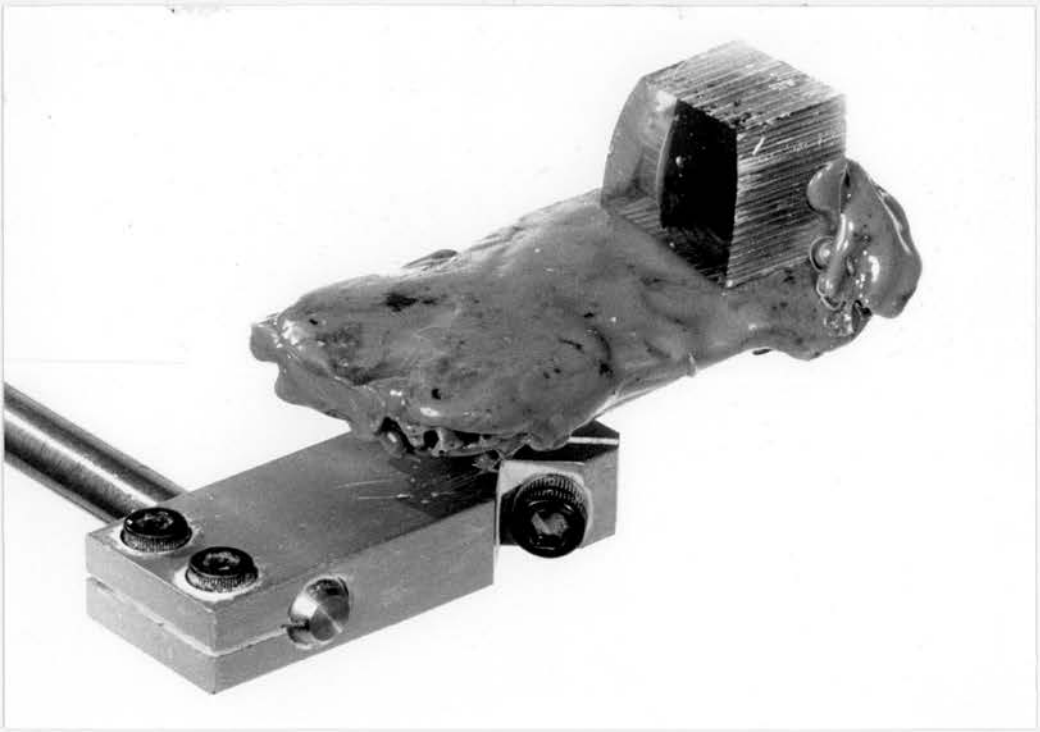


Fig 4. Microslice 2 (Cambridge Instruments Ltd) in annular configuration for the cutting of 1mm slabs. The specimen is fed into the cutting blade by a dampened counterbalanced see-saw.

POLYMASTER PROCESS.

1) Fixation of tissues in 70% alcohol for 24 hours.
2) Dehydration of specimens in four changes of absolute alcohol over 96 hours.
3) Infiltration in 100% resin until specimen has cleared.
4) Infiltration in three changes each of 24 hours in 95% resin / 5% plasticiser (dibutyl phthalate) at 37 degrees centigrade and 700mm vacuum.
5) Infiltration for 8 hours in 93% resin / 5% plasticiser / 1% Butinox 50 (catalyst) /1% inhibitor (1% hydroquinone in ethanol).
6) Embed in moulds and place in water bath (heat sink) until polymerisation has commenced.
7) Harden at 37 degrees centigrade for 24 hours, followed by a further 24 hours at 56°C.

---

diamond grit size and rotated at 200-300 rpm cutting each slab in approximately five minutes. The serial slabs were examined stereoscopically with a Zeiss binocular microscope using a transmitted light source. Viewing of the perfused vascular canals was improved by placing a drop of immersion oil on the upper face of the slab. Details of tissue vasculature were recorded using a 35mm camera attached to the sidearm of the microscope.

The material in the slabs was also studied histologically.

There were two methods employed to establish histological detail.

1)Surface staining of the slab: The surface of the slab was first cleaned with alochol. A few drops of 0.25% Toluidine blue were placed on one surface of the slab for two minutes,then the slab was rinsed in distilled water. The slab was then placed on a microscope slide stained surface upper most and viewed.

2)Cutting histological sections: The slabs were re-embedded in resin,the new block trimmed with the bandsaw and 5u sections cut. A heavy duty microtome (Polycut,Reichert-Jung) with a tungsten carbide knife was used to cut the sections. Sections were stained with haematoxylin and eosin, Masson Goldner trichrome and by the von Kossa method for calcium.

RESULTS.

Initially, problems occurred due to premature polymerisation of resin and resin/cellosolve mixtures. This was considered to be caused by the formation of peroxides in the cellosolve. Premature polymerisation was reduced by agitating solutions, keeping solutions cool, changed regularly and out of direct sunlight. The specimen in resin/cellosolve solutions were in sealed containers. These containers were agitated on a bed of revolving rollers designed for blood tubes.

There was no premature polymerisation of the plastic resin when alcohol was substituted for cellosolve during processing.

It had been noted in this laboratory that when bone specimens were processed in Polymaster resin the tissue became translucent.

The degree of tissue impregnation with Polymaster resin was assessed by the clearing of the skeletal tissue. Clearing varied between 24 hours and five days depending upon the specimen size. When the cartilaginous epiphysis was translucent then there was sufficient resin infiltration to proceed with the next stage of processing.

Both the cartilaginous epiphysis and physis cleared suffiently to allow accurate visualisation of the perfused vascular canals. The highly vascular marrow tissue in the diaphysis was poorly cleared by the resin. The vascular loops of MVs which were below the cartilaginous physis were well defined. Using the described technique the whole bone extremity was embedded in resin before cutting. There was therefore no leakage of perfusate from

cut vessels.

The surface staining of 1mm slabs was a rapid way of establishing both basic cellular morphology in the slabs and some of their detail, such as the patency of non perfused vascular canals. It was only where this technique had failed to provide enough information that slabs were re-embedded, sections cut and stained. The stained sections demonstrated excellent preservation of histological detail, which could be examined with reference to the information gained from the microangiographic study of the same material.

DISCUSSION

Polymaster resin was suitable in the preparation of perfused skeletal tissue for vascular and histological study.

The use of 70% alcohol as a fixative before absolute alcohol prevented rapid dehydration of the tissue and reduced the shrinkage of the cartilaginous skeletal components. Premature polymerisation of polymaster resin was a problem identified by Thorp et al (1985) and was due to cellosolve formed peroxides which were a resin catalyst. In the present study cellosolve was replaced by alcohol and this prevented the inadvertant introduction of peroxides which could act as catalysts for resin polymerisation.

The clearing of skeletal tissues in Polymaster resin was considered comparable to specimens prepared in Spalteholz fluid. The method of Polymaster resin embedding produced consistent results with a wide range of sizes of specimen. The procedure was shown to preserve histological detail, and all the resin slabs containing tissue were suitable for the subsequent preparation of histological sections. Processing of entire bone extremities prevented the leakage of perfusate from sectioned vessels, which was a problem identified by Rubin (1964). Cutting 1mm serial slabs from entire bone extremities enabled the entire vascular pattern to be established. Histological sections produced from 1mm slabs were not distorted and could be examined with reference to photographic records of their vasculature.

EXPERIMENT 3: Ad libitum fed S line fowls.

INTRODUCTION

The extremities of the developing long bones in vertebrates are made up of an epiphysis, physis and metaphysis. The epiphysis is initially cartilaginous, but will eventually become ossified and can contain secondary ossification centres. The surface of the epiphysis is covered in articular cartilage or perichondrial tissue. The physis or growth plate is situated below the epiphysis and is responsible for growth in length of the long bone. The metaphysis, lying below the physis, is continuous with the diaphysis and its function is the formation of metaphyseal trabecular bone.

The epiphysis contains epiphyseal vascular canals (EVCs), some of which penetrate the physeal cartilage (PEVs). The physis is also penetrated by vessels from the metaphyis, the MVs (MVs). For the purposes of the present study however different regions in the bone extremities are defined by the extent of their vascularity. The metaphysis is considered to extend to where the tips of the MVs made contact with the physeal cartilage. The physis lay between the epiphyseal hyaline cartilage and the MVs. There are tongues of physeal cartilage between the MVs, however as this is principally a vascular study, this zone was considered to be part of the metaphysis.

The object of this experiment was to establish the vascular

pattern of the bone extremities of the three principal long bones in the pelvic limb of the normal developing avian skeleton. For this experiment birds of the S line strain were chosen which are derived from the White Leghorn. Leghorn fowls are bred exclusively for egg production and are almost without skeletal abnormality (Reiland et al ,1978a).

CARTILAGE CANALS.

The structure and function of the vessels in the extremities of growing bones has been investigated, by many workers, in a variety of species. Harris (1929) studied the growing long bones of man. He identified vessels in the ossified epiphysis and metaphysis, but considered the physis to be avascular. Brodin (1955) found that when fluorescent substances were injected intravenously they were rapidly taken up by the cartilaginous epiphysis of the rabbit. This suggested that the cartilage contained a system of vascular canals. Heraldsson (1962) investigated the canals in the cartilaginous epiphysis of the human foetus. He considered that because of the anatomical isolation of cartilage canals they should be considered to function as end vessels.

The cartilage canals in the epiphysis do not all share the same function. The bone extremities from mid-gestation sheep foetuses were studied by Stockwell (1971). He divided the cartilage canals of the epiphysis into two groups, terminal and conducting. This distinction was based upon morphological

differences between canals. The terminal canals were "glomeruli" like with only a thin layer of endothelium between the blood vessels and the cartilage. In the conducting canals there were three layers between the vessels and the cartilage matrix. These layers were endothelium, polymorphic cells with a pale cytoplasm and dense fibriller tissue.

The cartilage canals grow and divide as the epiphysis increases in size. Cartilage canals were studied in the human foetus by Wang (1975). He noted numerous vessels in the epiphyseal hyaline cartilage of the humerus, and during growth these cartilage canals underwent a series of regular changes. They developed into a network of branching and subdividing vessels in the cartilage. This statement agrees with Levene (1964), who described that active growth of the cartilage canals occurs rather than a passive incorporation of vessels into cartilage.

Branches from EVCs. which supply the physeal cartilage, are termed penetrating epiphyseal vessels (PEVs). The vascularity of the epiphysis in the ulna of the rabbit was studied by Spira et al (1963) and Spira and Farin (1967). Vessels (PEVs) from the cartilaginous epiphysis were described as invading the physis as far as the hypertrophic zone of chondrocytes. When the epiphysis was fully ossified there were no vascular canals in the physeal or articular cartilage. Brighton (1978) described vessels (PEVs) in the epiphysis as arborising in a rake like fashion to supply the physis. Physeal vessels (PEVs) in the rabbit were considered to be in direct contact with the cartilage matrix (Trueta and Little, 1960).

One of the main functions of the EVCs is the maintenance of the epiphyseal hyaline cartilage, which is borne out in descriptions of the canals. Cartilage canals are blind ending and contain a large number of thin walled vessels resembling wide capillaries with the occasional artery (Kugler et al, 1978). Wilsman and van Sickle (1972) provided a similar description of epiphyseal vascularity. They stated that the canals contain an arteriole, venule, loose connective tissue and perivascular capillaries. The canal terminates in a capillary glomerulus The canals were described as end arteries with no collateral circulation. In studies of the human humerus, Heraldsson (1962) concluded that, the function of cartilage canals is to supply cartilage too large to be nourished by diffusion of nutrients through the substance of the matrix. A nutritive function is ascribed to the canals in the epiphyseal hyaline cartilage of the dog by Wilsman and van Sickle (1972). At birth in the dog there were subarticular canals but these soon disappeared. Their disappearance was attributed to a significant nutritional effect of the synovial fluid on the subarticular cartilage brought about by weight-bearing. The spacing between the cartilage canals in the dog remains constant during growth at 1 - 1.5mm (Harvey and van Sickle, 1971). The metaphyseal side of the physis is eroded by MVs prior to ossification. Lewis (1956) described MVs in the rabbit and human embryo as being derived from the nutrient artery or metaphyseal arteries. The MVs had saccular dilations where they met the growth plate. The MVs in foetal rats and also those at term were described as juxta-epiphyseal sinusoidal networks

which were dilated and varicose (Brookes and Landon, 1964). The MVs were an end arterial system with no break in the endothelium. Brookes (1963) provided a similar description of the MVs in the human foetus. He also stated that there were no transverse anastomoses between MVs which appeared as tufts of sinusoids dispersed across an invasion front, each front being supplied by an arteriole. Baltadjiev (1981) examined the histology of the MVs in the new born infant. He described two types of MVs which penetrated the growth cartilage. These were ordinary capillaries and sinusoidal type vessels The sinusoidal vessels were irregular in outline and had uniform bag-like dilations. Two types of MVs were also described by Brighton (1978).

Ultrastructural studies by Cameron (1961) demonstrated that the MVs in conjunction with osteoclasts absorbed the calcified matrix of the physis in the tibia of the rat. The MVs in cattle were studied by Cerny (1983). He stated that MVs resembled sinusoidal blood capillaries terminating as blind apices reminiscent of vascular buds. The walls of these capillaries were the attenuated process of endothelial cells. Micro-haemorrhages containing erythrocytes and plasma occupied the perivascular space. There were both free erythrocytes and capillaries in direct contact with the ground substance of the cartilage.

Controversy exists as to the presence or absence of vascular connections across the physis, between the epiphyseal and metaphyseal circulations. The absence of such connections has been definitively stated (Harvey and van Sickle, 1971; Baltadjiev, 1981; Lewis, 1956). Other workers have reported

transphyseal anastomoses (Brookes, 1958b; Gray and Gardner, 1969 and Gardner and Gray, 1970; Heraldsson, 1962.). Brookes (1963) recognised transphyseal vessels in the human foetus, but considered that they crossed from the metaphysis to the epiphysis. Smutts (1978) examined the cervical vertebra in the calf. He described anastomoses as occurring between the epiphyseal and MVs. The foal also has vessels crossing from the epiphysis to the metaphysis in the distal radius and metacarpus (Firth and Poulos, 1982). A temporary nutritive function is ascribed to these vessels. Transphyseal vessels persist postnatally in the foal but are only identified in the foetus of man and rabbits (Firth and Poulos, 1983). This is considered to be due to the slower development of the metaphyseal arteries in the horse. The occurrence of transphyseal vessels in the long bones of the young pig were considered to be abnormal (Bullough and Heard, 1967). In a study of pigs, from birth to fifteen days of age, 67 out of 175 bone extremities contained transphyseal vessels (Hill et al, 1985). The majority of penetrating epiphyseal vessels terminated 1/2 to 2/3rds of the distance across the physis, adjacent to the zone of hypertrophic chondrocytes.

The physis is encircled by a ring of perichondrial tissue and is a fibrous structure richly supplied by several arteries (Brighton, 1978). The functions of the perichondrial ring are principally to enable the physeal disc of cartilage to increase in size, provide mechanical support to the physis and to act as a local vascular supply. The perichondrial ring is considered to include the "perichondrial ring of La croix", the fibrous

structures of the periphery of the growth plate and the "groove of Ranvier". In the present study, no distinction is made between these three histologically distinct regions of the perichondrial ring.

The epiphysis of the fowl was considered to be wholly cartilaginous and to contain no centres of ossification (Haines and Mohuiddin, 1962). This report was superceded by Wise and Jennings (1973), who noted secondary ossification centres do not invariably occur at the ends of the avian long bone. Where they do occur, as in the proximal and distal tibiotarsus and the proximal tarsometatarsus, they are initially smaller than in mammals and are surrounded by hyaline cartilage.

In the long bone in the embryonic chick chondrogenesis and osteogenesis is initially at the centre of the shaft. A system of cylindrical canals then forms in the epiphysis. Concurrently finger shaped outgrowths from the marrow penetrate towards the cartilage extremities of the diaphysis (Fell, 1925). Wolbach and Hegsted (1952) described the vascularity of the bone extremities in the growing chick. The cartilaginous epiphysis and physis both contained blood vessels which were separate from the metaphyseal circulation. The cartilage canals were surrounded by loose connective tissue continuous with the perichondrium. The growth plate of the turkey was investigated by Wise and Jennings (1973). The presence of PEVs as parallel-sided tunnels distributed randomly through the proliferative zone of chondrocytes was described. There were approximately twenty cell columns between the PEVs. There was a layer of avascular cartilage approximatly

fifteen cells deep between the MVs and PEVs. They also noted the similarity of the zones in the turkey growth plate to mammalian growth plates.

The cartilage canals in the ulna of the domestic fowl were described by Beaumont (1967). There were from the metaphysis uniformly spaced vascular loops invading the physeal cartilage. Occasionally the arching vessels in the epiphysis formed loops of blood vessels which descended vertically into the physis, with no transphyseal vascular connections or anastomoses between epiphyseal vessels.

The long bone extremities of the fowl from the embryo to the adult bird were studied by Lutfi (1970a). PEVs were present from day thirteen in the embryo. The physis of the fourteen day old embryo contained definitive reserve, proliferative and hypertrophic zones of chondrocytes. In the young chick there were regularly spaced PEVs. The EVCs became wider and ramified more extensively with increasing age. There were no anastomoses between branches and vessels of separate canal systems. EVCs were widely spaced and undergoing an "endarteritis obliterans" by sixteen weeks of age. The avian physis is penetrated by complexes of thin walled MVs (Howlett, 1980). In an investigation of rickets in the fowl Lacey and Huffer (1984) initially studied the vascular anatomy of the normal proximal tibiotarsus. They described the growth disc (physis) as being penetrated by vascular loops from the epiphysis the PEVs. The metaphyseal side was penetrated by the terminal portions of the nutrient and metaphyseal vascular systems, referred to collectively as MVs.

The chondroepiphysis and growth plate in the four week old chick were well vascularised by cartilage canals and arrays of PEVs. The PEVs were described as being supplied by junctional canals which overlay the physis. Ultrastructural studies suggested that the highest concentration of nutrients would be found in the capillaries near the bottom of the canal (Lacey and Huffer, 1984).

At hatching, cones of cartilage were present in the ends of the long bones of the turkey (Wise and Jennings, 1973). The cones were penetrated by blood vessels from the epiphysis. When a full array of MVs had formed across the metaphysis there were no epiphyseal vessels descending into the remnants of cartilage, which were still present below the metaphysis. In the human foetus PEVs have been described as branching into the metaphysis (Wang 1975).

Levene (1964) studied the cartilage canals in the cartilaginous epiphysis of the proximal tibia in sheep, goats, rabbits and man. He found that the cartilage canals in all members of the same species followed a similar pattern. The pattern was basically unchanged from early foetal life until an ossified epiphysis had formed. The pattern became more complex as the system of canals increased in size with growth. The pattern of arterial anatomy in the chick embryo limbs is constant between different individuals.

The purpose of the present study was to establish and investigate the pattern of cartilage canals in the extremities of the avian long bone.

MATERIAL AND METHODS.

One hundred and twenty four chicks (sixty two of each sex) were reared from day old in deep litter floor pens. The environment was controlled at a steady temperature and a regular lighting pattern of fourteen hours light and ten hours dark. The birds were fed ad libitum a commercial starter ration (23% protein, 3000kcal ME) until four weeks of age followed by a grower ration (19% protien, 3000kcal ME)*. The birds were all weighed weekly.

Eight birds (four male and four female) were killed at day old. Thereafter, birds were killed at two, five, seven, nine, 14, 21, 28, 42, 70, and 140 days. After killing the birds were routinely weighed. Angulations of the intertarsal joint were recorded. After dissection the pelvic appendicularl skeleton was radiographed in a Faxitron 804 using Kodak X-Omat RP film in a Kodak X-Omatic fine screen plate. Both AP and lateral views were taken. Estimates of torsion were recorded for the three long bones, by comparing the transverse axis of the proximal and distal articular surfaces (Duff and Thorp, 1985a and 1985b). The post mortem details, of age, sex, weight, intertarsal angulation, long bone torsion and long bone length, from each bird are recorded in Appendix 1.

Four specimens from each kill were processed in Polymaster resin, and the other four were stored in 10% BNF. The Polymaster blocks containing the proximal and distal femora, proximal and distal tibiotarsi and the proximal tarsometatarsi were

* Further details of diets in appendix 5.

subsequently cut into slabs and examined.

The epiphyseal hyaline cartilage contained epiphyseal vascular canals (EVCs), which branched through the cartilage. The EVCs terminated either in the physis (growth plate cartilage) as penetrating epiphyseal vessels (PEVs) or in the epiphyseal hyaline cartilage as blind ending capillary loops. All EVCs were end arterial systems and there were no vascular connections between individual arborising EVCs.

The term principal EVC has been used to denote EVCs with a number of characteristics:

1) The principal EVCs followed a regular path through the epiphysis.
2) The principal EVCs were of greater diameter than other EVCs
3) The principal EVCs did not directly form PEVs but branched to form EVCs which then divaricated through the cartilage or formed PEVs.

The even contour to the expanding EOCs, especially in the distal tibiotarsus, was sometimes interrupted by local infolds of epiphyseal hyaline cartilage. These invaginations of cartilage into the ossified epiphysis always contained a cartilage canal to the EOC. There were no specific junctional canals between the epiphysis and physis in any of the bone extremities examined.

The physis was uniform in thickness. Evenly spaced, parallel

sided MVs, were invading the physeal cartilage. There was an avascular zone of hypertrophied chondrocytes between the descending PEVs and ascending MVs. In the newly hatched chick there was a cartilage cone occupying the metaphysis, which was penetrated by elongated transphyseal PEVs. The transphyseal PEVs disappeared with the formation of an array of MVs across the metaphysis. In Von Kossa stained sections there was no calcium deposition in the cartilage prior to the formation of an array of MVs. Epiphyseal ossification centres (EOCs) only occurred in the proximal tibiotarsus, distal tibiotarsus and the proximal tarsometatarsus. In the proximal femur,distal femur and the proximal tibiotarsus there were many similarities between the pattern of vascularity in the fowl and other species, This was not true in the bone extremities of the hock joint, because tarsal elements were incorporated into the developing distal tibiotarsus and proximal tarsometatarsus.

Bone extremities of the pelvic appendicular skeleton were examined in the order of proximal femur, proximal tibiotarsus, proximal tarsometatarsus, distal femur and distal tibiotarsus. There were marked differences in the vascularity of the different bone extremities. Each bone extremity is described separately.

There was no EOC in the proximal femur. The epiphyseal hyaline cartilage extended across the entire proximal extremity of the femur, incorporating the femoral head and trochanter. The origin of the vascular supplies to the EVCs of the proximal femur could be used to divide the EVCs into groups. A diagram of the cross section of the proximal femur (Fig 5) represents the EVCs and their origins. Individuals of the same age demonstrated a similar pattern in the area supplied by an EVC or group of EVCs. These areas are delineated in Fig 6.

EVCs originated from:

1) The perichondrial ring.
2) The intracapsular retinacular tissue.
3) The joint capsule and associated connective tissue.
4) The extracapsular retinacular tissue.
5) The capital femoral ligament.

The perichondrial ring encircled the proximal femoral growth plate. The main vascular supply to the perichondrial ring was from the mid-lateral, mid-cranial and mid-caudal femur. It was from these three regions that the majority of perichondrial EVCs originated (Fig 6I).

The caudal perichondrial ring vessel formed either two or three EVCs which supplied the caudal trochanter and the caudolateral femoral head (Fig 7). The lateral femoral perichondrial ring vessels were the source of EVCs to the

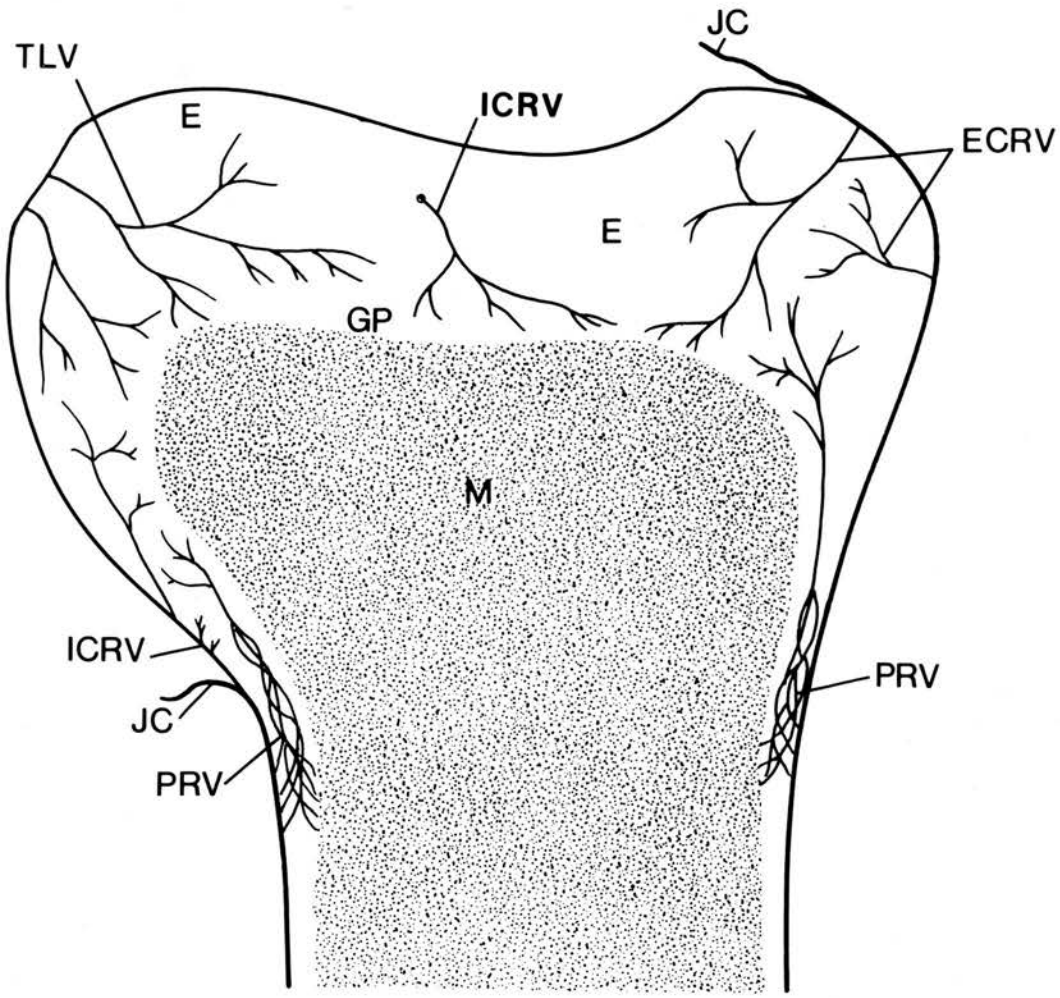


Fig 5. Coronal section of the proximal femur demonstrating the EVCs and their sites of origin.

ECRV: originating from the extracapsular retinacular vessels.
GP: growth plate or physis.
ICRV: originating from the intracapsular retinacular vessels.
JC: joint capsule.
PRV: perichondrial ring vessels.
TLV: teres ligament vessels.

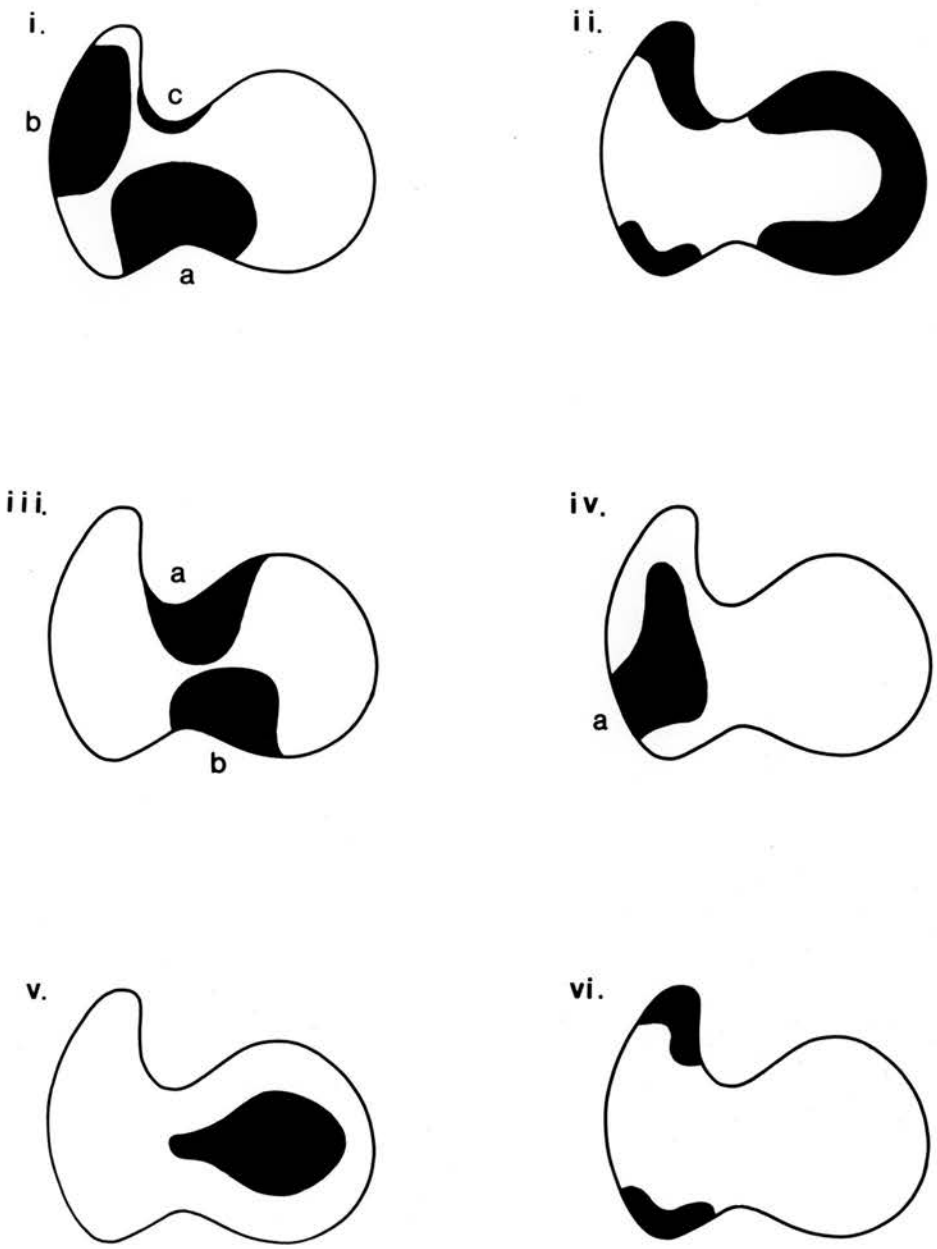


Fig 6. Diagrammatic representation of the left proximal femur in the fowl. The shaded areas represent the region supplied by a specific group of EVCs.

Ia EVCs from the caudal perichondrial ring.
b EVCs from the lateral perichondrial ring.
c EVCs from the cranial perichondrial ring.
II EVCs from ICRVs.
IIIa EVCs from the large retinacular vessel.
b EVCs from a branch of the large vessl to the caudal perichondrial ring.
IVa EVCs from ECRVs on the lateral aspect of the trochanter.
V EVCs from vessels in the teres ligament.
VI EVCs from vessels in the joint capsule and connective tissue.

trochanter (Fig 8). The cranial perichondrial ring vessels only rarely formed EVCs. These EVCs which did not form PEVs, occurred most frequently in day old and six to ten week old birds.

The intracapsular retinacular tissue contained blood vessels. These retinacular blood vessels originated as vessels in the joint capsule and perichondrial ring. Vessels from the retinaculum of the cranial femoral head extended onto the joint surface of the lateral femoral head. EVCs from the retinacular vessels supplied PEVs to the periphery of the femoral head,and to the margins of the cranial and caudal trochanter (Fig 6Ⅱ).

The retinacular vessels of the craniolateral femoral head were supplied by a large vessel from the extracapsular soft tissue. A branch from this vessel(Fig 9) continued as an EVC to supply the craniolateral femoral head, area (a) in Fig 6Ⅲ.

The caudolateral femoral head (capitus) was supplied by an EVC which was a branch from the vessel to the caudal perichondrial ring (vessel c in Fig 10). This vessel branched to supply the area (b) in Fig 6Ⅲ. The lateral surface of the trochanter was covered in a fine reticular network of vessels. The epiphysis of the mid-and caudal zenith (apex) of the trochanter was supplied by EVCs originating from these retinacular vessels (Fig 6Ⅳ).

The capital femoral ligament (teres ligament) was the origin of EVCs to the central femoral head. Approximately six EVCs radiated out from the fovea (Fig 6Ⅴ & Fig 11).

Small EVCs penetrated the epiphyseal hyaline cartilage of the cranial and caudal trochanter.They were the terminal branches of vessels in the joint capsule and associated connective tissue (Fig

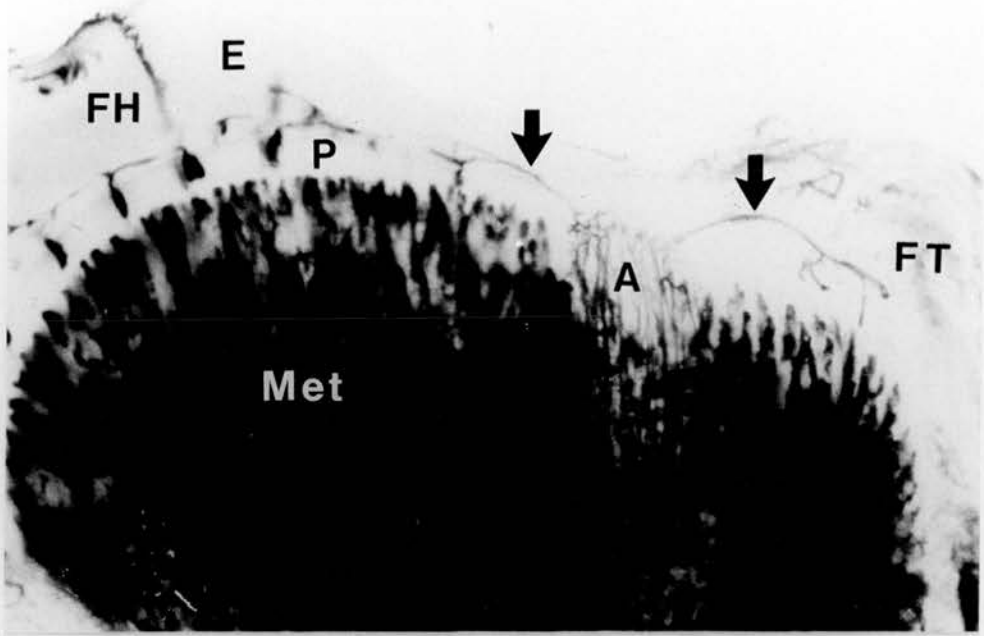


Fig 7. The right proximal femur of a 28 day old S line. The caudal perichondrial ring vessels (A) supply EVCs (arrowed) to the cartilage of the femoral head and trochanter.1mm slab x25.

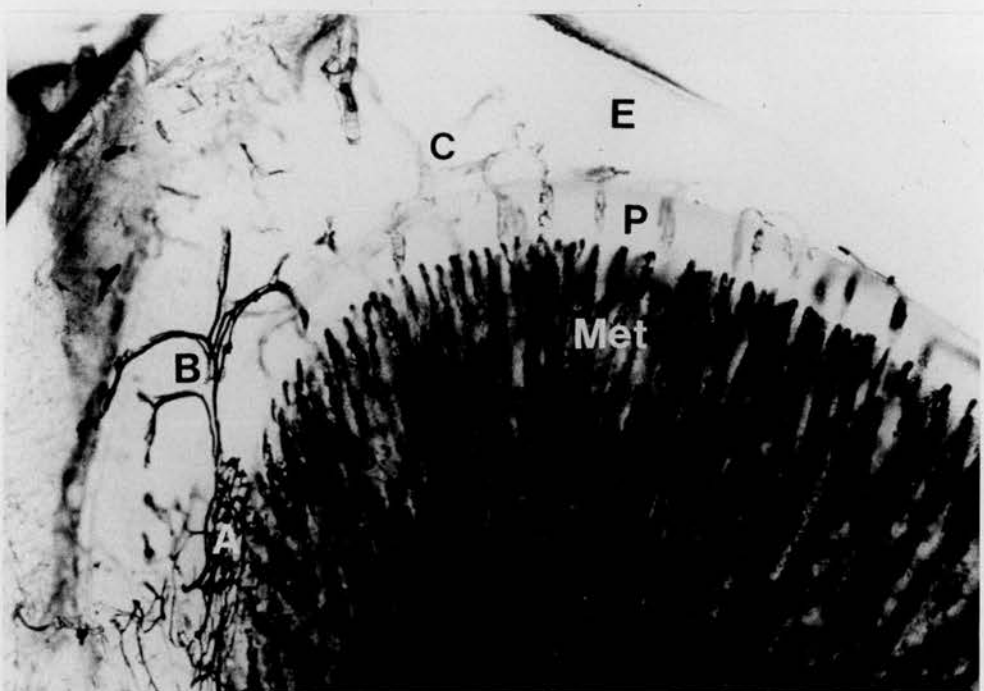


Fig 8. The trochanter from the right proximal femur of a 28 day old S line. The lateral perichondrial ring vessels (A) supply an EVC (B) which extends into the trochanteric cartilage. The retinacular vessels on the lateral trochanter supply an EVC (C) which extends across the apex of the trochanter forming PEVs.1mm slab x16.

Fig 9. A slab from the left proximal femur of a 42 day old S line. The plexus of cranial perichondrial vessels (a) do not form EVCs The large retinacular vessel (arrowed) is on the surface of the articular cartilage. This vessel forms EVCs which supply the cranio-lateral capitus. 1mm slab x16.

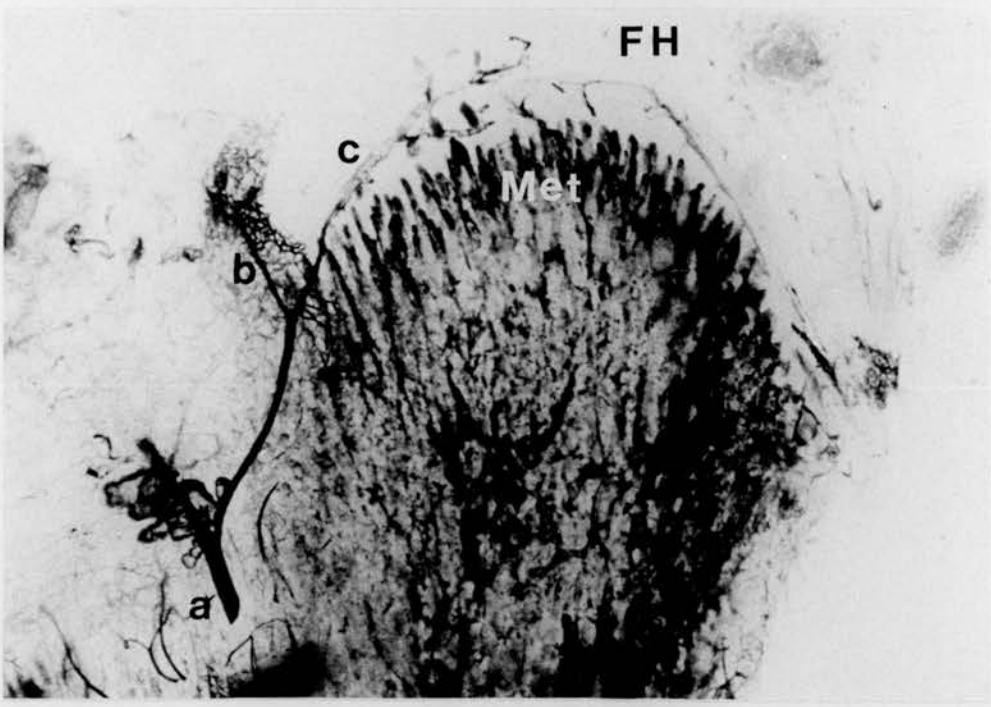


Fig 10. The caudal aspect of the right proximal femur of a 14 day old S line. The large vessel (a) divides to supply the perichondrial ring (vessel b) and an EVC (c) to the femoral head. 1mm slab x25.

6VI).

Changes with age

Day Old

The diaphysis and metaphysis of day old specimens contained a cartilage core. PEVs were elongated and crossed the physis to penetrate the cone (Fig 12) and distally connect with the large medullary vascular canals. Frequently these transphyseal PEVs originated from the caudal perichondrial ring EVCs.

In some specimens the EVCs from the capital femoral ligament were poorly developed. In these femoral heads there was a more extensive supply of EVCs from the retinacular vessels. The caudal trochanter was supplied by three EVCs from the lateral retinacular vessels.

The cranial trochanter was supplied by EVCs from the perichondrial ring vessels of the mid-lateral trochanter. Vessels originating from the joint capsule of the cranial and caudal trochanter formed short EVCs but no PEVs.

Branching MVs were present around the periphery of the growth plate.

Day two

The MVs had arborised to form a complete array across the metaphysis below the physeal cartilage (Fig 13). The physis was no longer crossed by transphyseal PEVs. The cartilaginous cone was now smaller and confined to the diaphysis. From the

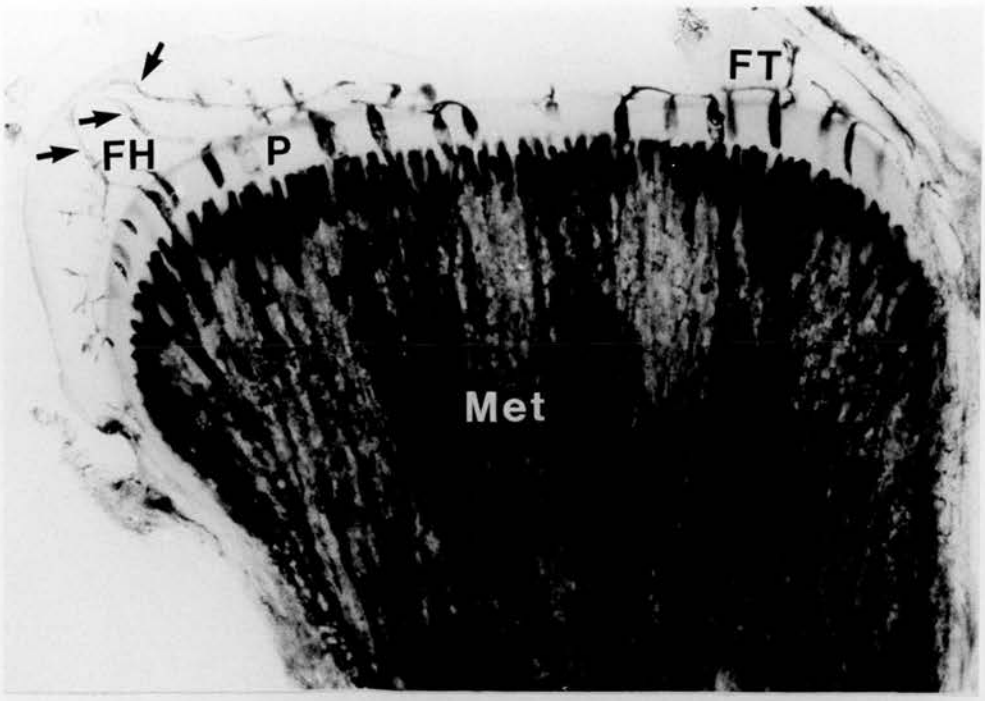


Fig 11. The right proximal femur of a 14 day old S line. Three EVCs (arrowed) originating from vessels in the teres ligament extend through the epiphysis to form PEVs.1mm slab x25.

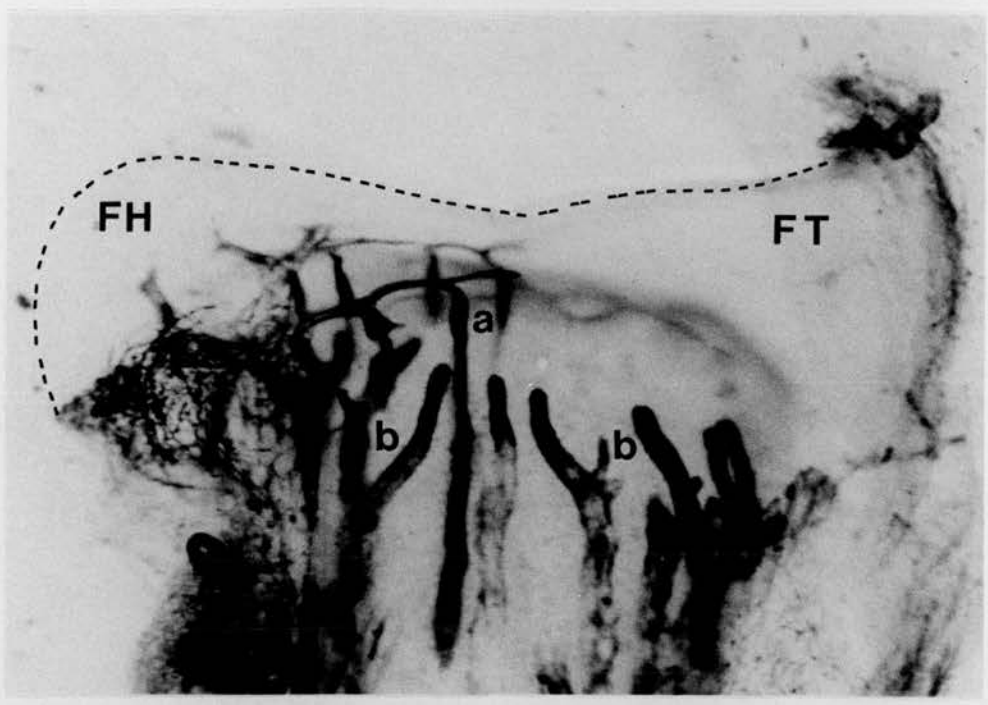


Fig 12. The right proximal femur of a day old S line. PEVs (a) from the caudal perichondrial vessels, extend down into the cartilage core. MVs (b) branch around the periphery of the the metaphysis. The cartilaginous epiphysis is mainly avascular.1mm slab x40.

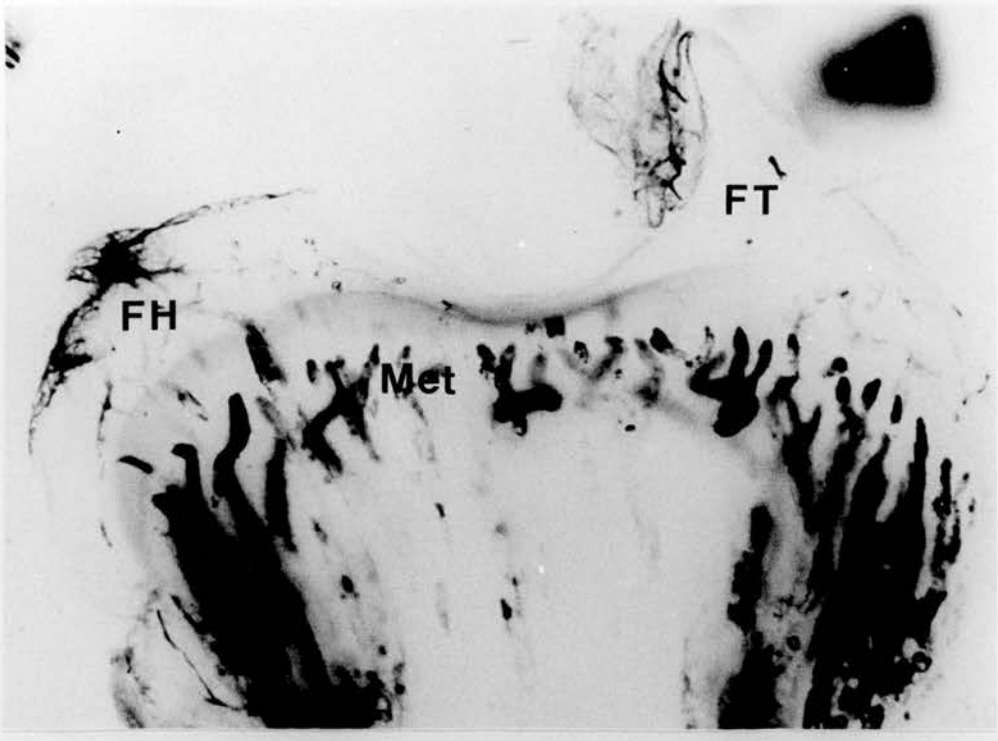


Fig 13. The proximal femur from a 2 day old S line. MVs form an irregular array across the entire metaphysis below the physis.1mm slab x40.

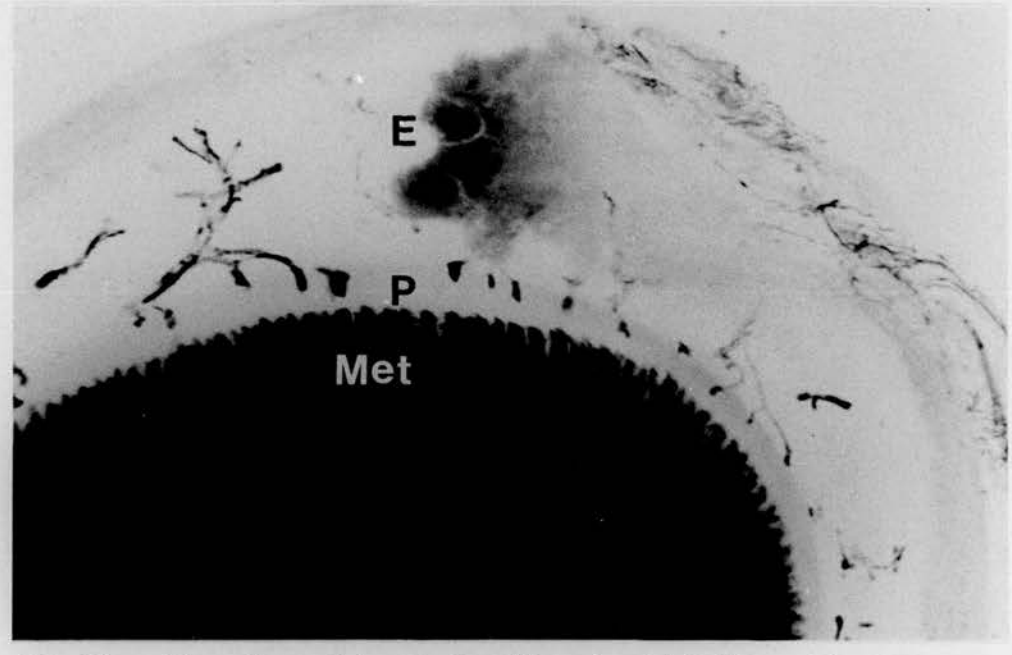


Fig 14. The femoral head of a 10 week old S line. The PEVs are short and widely spaced. There is a reduction in the number and extent of the EVCs.1mm slab x25.

diaphysis, wide medullary vascular channels invaded proximally along the path of the narrower canals, which had been formed previously by the transphyseal PEVs.

The EVCs from the joint capsule of the cranial trochanter and surrounding tissue now formed PEVs. In all the specimens there was a well developed supply of EVCs from the capital femoral ligament. The PEVs were now more evenly spaced across the growth plate.

Day seven

The cartilaginous diaphyseal cone was no longer apparent. The caudolateral femoral head was now supplied by a branch of the vascular supply to the caudal perichondrial ring. This vessel frequently trifurcated in the cartilaginous epiphysis to extend medially, cranially and craniolaterally. The EVCs from the extracapsular retinacular vessels on the lateral aspect of the trochanter (usually five in number) extended cranially to supply the cranial trochanter with PEVs.

Day 28

EVCs from the caudal perichondrial ring extended laterally to supply the caudal trochanter. Retinacular EVCs from the lateral trochanter supplied EVCs to the cranial and mid-trochanteric crest. The cranial margin of the trochanter was supplied by cranial trochanteric EVCs which originated from the cranial joint capsule.

The vascular supply to the retinacular tissue and

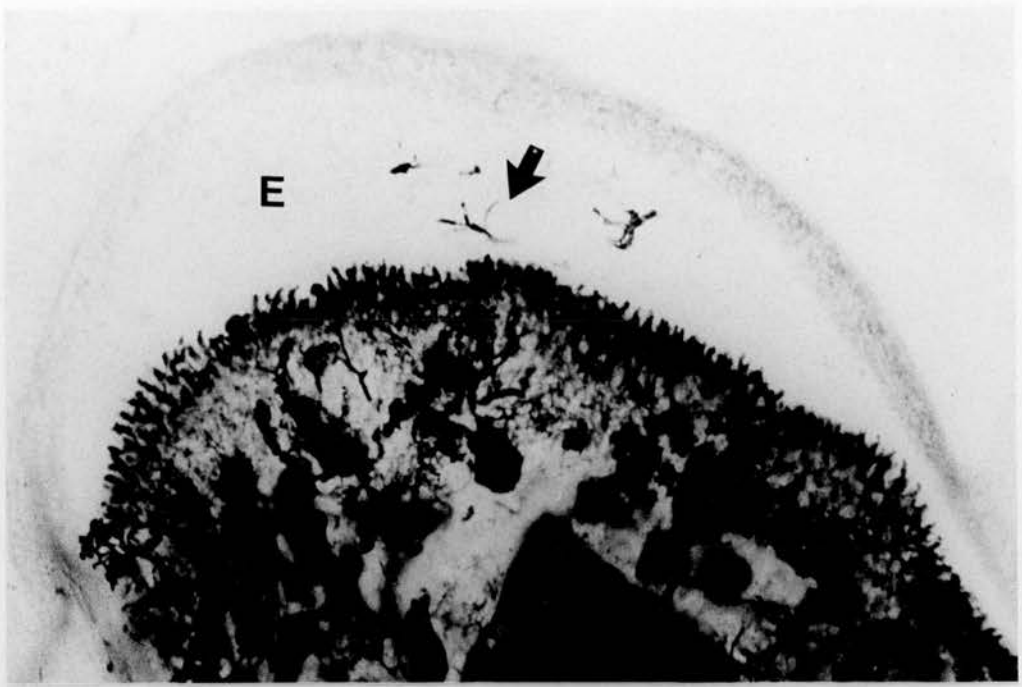


Fig 15. The femoral head from a 15 week old S line. There are no PEVs. There is an EVC "ghost" (arrowed). The MVs have extended through the physis and are invading the cartilaginous epiphysis.1mm slab x16.

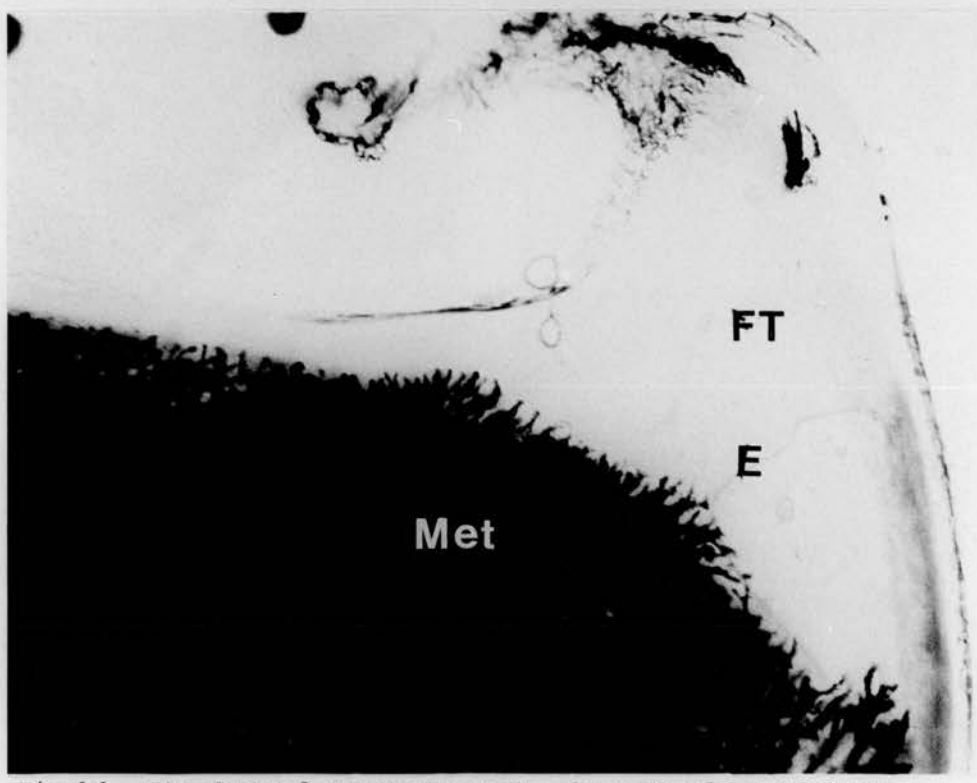


Fig 16. The femoral trochanter of a 15 week old male S line. The cartilaginous epiphysis is avascular.1mmslab x20.

perichondrial ring was mainly from vessels on the cranial and caudal aspects of the femur. The perichondrial and retinacular tissues were more vascular in the cranial and caudal proximal femur.

Day 70

The PEVs in female birds were shorter, and there was a reduction in the apparent number of PEVs. The spacing between the PEVs was greater (Fig 14). The EVCs were not as extensive as in the younger females. No such changes were apparent in any of the male specimens.

Day 105

There was a marked reduction in the number of EVCs. In some femoral heads there were no EVCs from the capital femoral ligament (Fig 15), but there were still EVCs from the peripheral perichondrial and retinacular vessels.

The caudal half of the trochanter was completely ossified apart from a thin surface layer of cartilage. The cranial trochanter was mainly avascular hyaline cartilage (Fig 16). MVs were now arranged as branching vascular tufts, slowly eroding the remaining cartilage of the femoral head and trochanter.

Day 140

The remaining epiphyseal hyaline cartilage was avascular. The invading MVs formed an uneven boundary between it and the metaphysis.

Fig 17. The femoral head of a 10 week old S line contains an area of disturbed MV penetration (D). The PEVs above the defect are enlarged. 1mm slab x16.

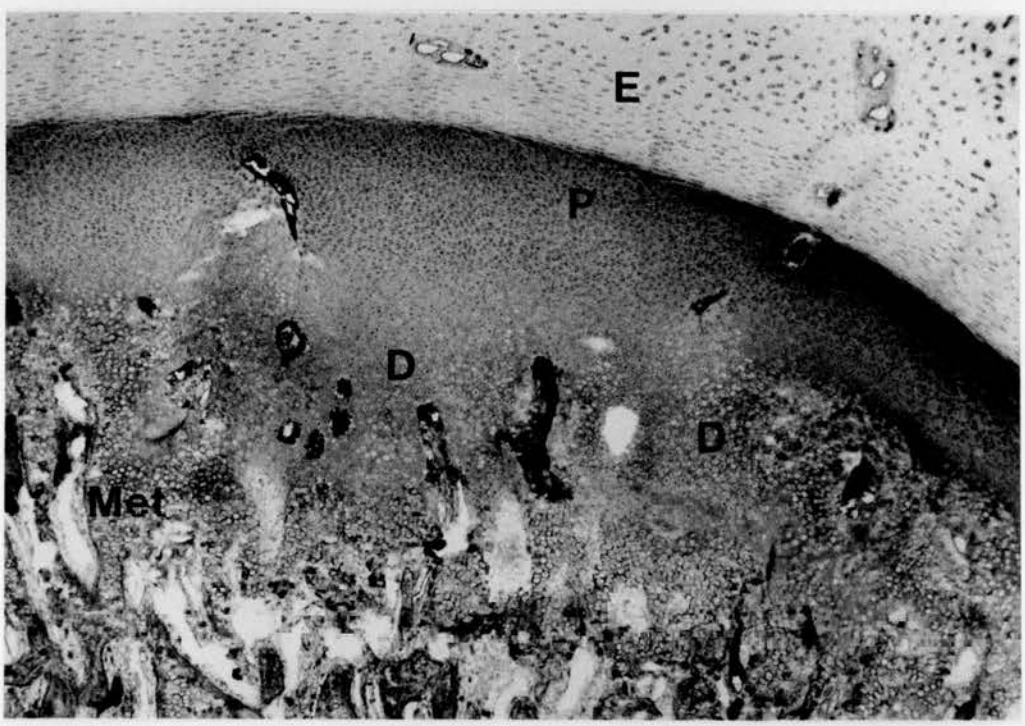


Fig 18. The same metaphyseal defect as in fig 17. There is an increase in the depth of the zone of prehypertrophied condrocytes at the site of disturbed endochondral ossification. H & E x40.

Vascular defects occurring during growth

A small number of the proximal femurs examined demonstrated focal areas of delayed endochondral ossification. Two types of lesion were identified.

In the first type there was a thickening of the physeal cartilage, causing avascular cartilage to extend down into the metaphysis (Fig 17). This lesion occurred in the central femoral head of two specimens and in the proximal femoral neck of another. All three were ten weeks of age. Histologically (Fig 18) these lesions were characterised by an increase in the thickness of the prehypertrophied zone of chondrocytes.

The second type of lesion occurred towards the end of growth. In a twenty week old specimen there was a delay in the invasion of the epiphyseal hyaline cartilage by MVs. The site of the lesion was in the cranial trochanter which was avascular but being revascularised by a branching EVC (Fig 19).

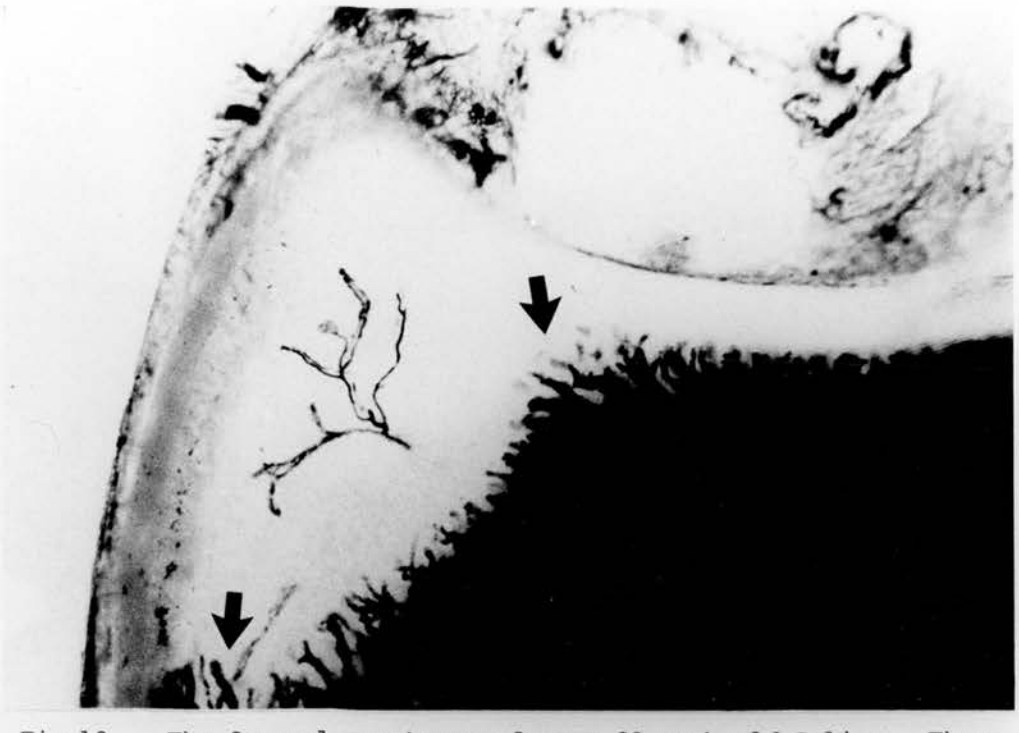


Fig 19. The femoral trochanter from a 20 week old S line. There is retention of epiphyseal hyaline cartilage. MVs (arrowed) are penetrating the epiphysis around the periphery of the retained cartilage. 1mm slab x16.

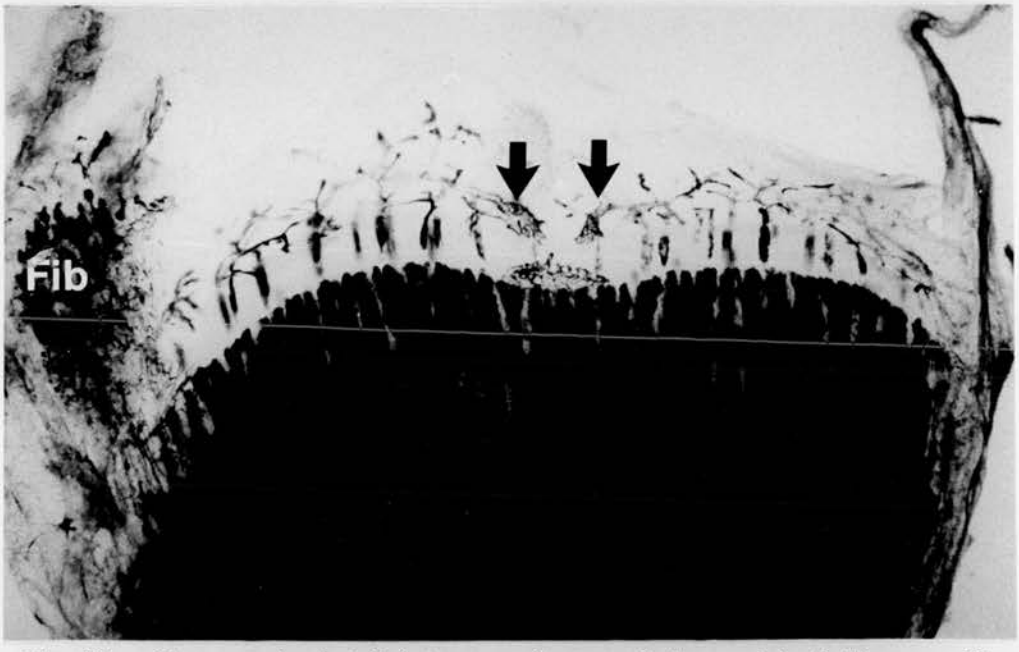
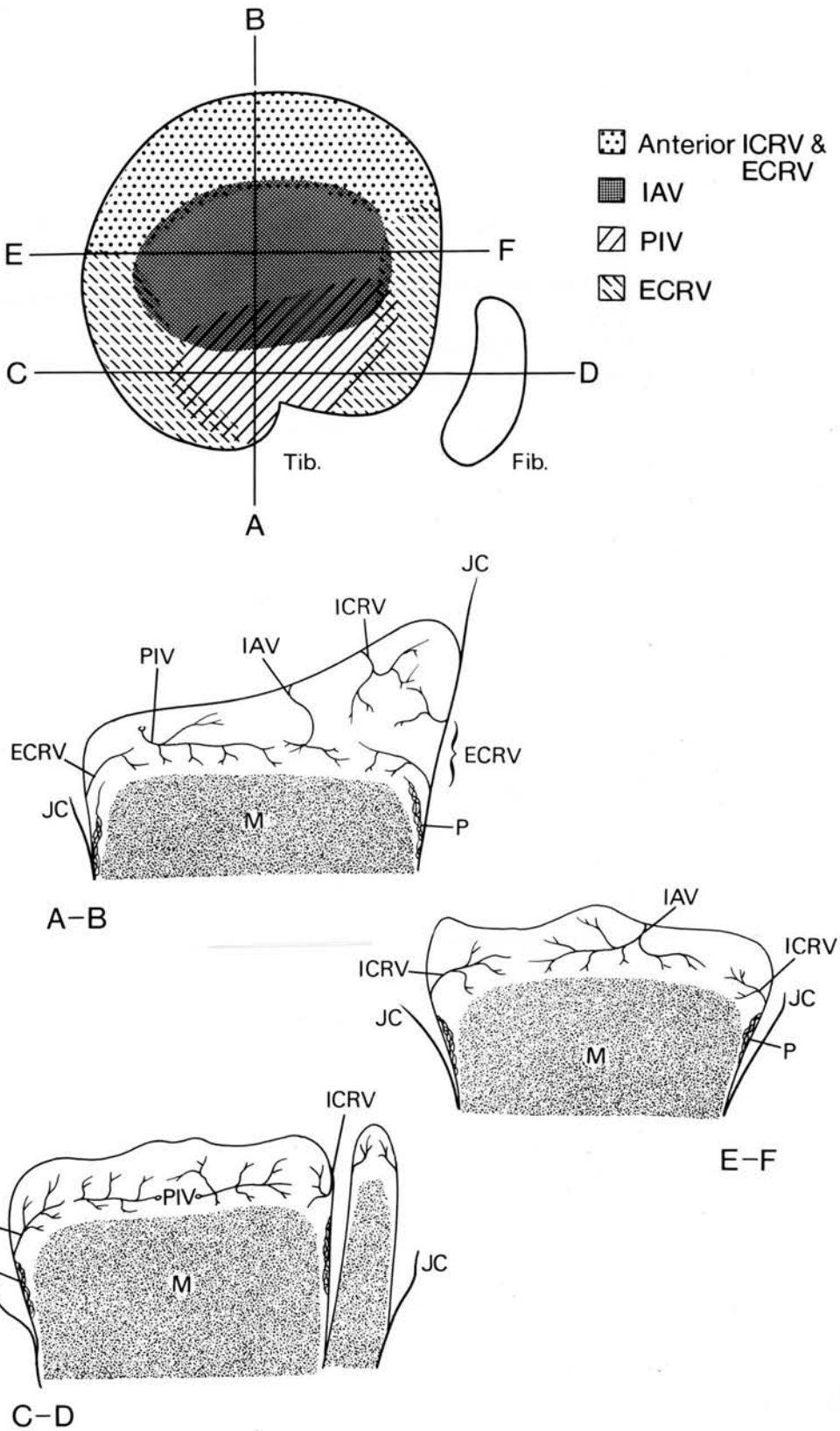


Fig 20. The proximal tibiotarsus from a 7 day old S line. The two principal EVCs (arrowed) that originate from the penetrating intercondylar vessel branch through the condyles. 1mm slab x25.


B
E
F
C
D
A
Tib.
Fib.
Anterior ICRV & ECRV
IAV
PIV
ECRV
JC
ICRV
PIV
IAV
ECRV
ECRV
JC
M
P
A–B
IAV
ICRV
ICRV
JC
JC
M
P
E–F
ICRV
PIV
ICRV
P
JC
M
JC
C–D

PROXIMAL TIBIOTARSUS

The vascularity of the proximal tibiotarsus conformed to a general pattern, which was modified during three different stages of growth. In specimens of the same age there was only minor variation in the vascular pattern.

The growth stages which modified vascularity were:

1)The formation of a fully functional growth plate.

2)The development of the epiphyseal ossification centre.

3)The cessation of growth.

General Vascular pattern.

The extent of the EVC systems and the area they supply in the proximal tibiotarsus is represented diagrammatically in Fig 21. The perichondrial ring around the periphery of the growth plate was poorly developed, with the perichondrial ring only forming EVCs in the intercondylar groove on the caudal aspect. There were vascular connections between vessels of the perichondrial ring, the retinacular vessels and MVs. The EVCs derived from the perichondrial ring did not form PEVs.

The soft tissue caudal to the tibiotarsus was the origin of a large vessel, the penetrating intercondylar vessel (PIV), which penetrated the epiphyseal hyaline cartilage in the groove between

the caudal aspect of the medial and lateral condyles. This vessel became an EVC which characteristically divided into medial and lateral branches (Fig 20). These branches were the main supply of condylar EVCs and PEVs.

Intracapsular retinacular vessels (ICRVs) on the medial, lateral and caudal aspects of the epiphysis supplied the EVCs and PEVs to the peripheral of the cartilaginous epiphysis (Figs 21). The EVCs formed by the caudomedial ICRVs were more extensive and extended deeply into the medial condyle. The lateral ICRVs were fewer in number, smaller in size and supplied less extensive systems of EVCs. They supplied the cartilaginous epiphysis and physis of the tibiotarsus adjacent to the fibula.

The cranial aspect of the cartilaginous epiphysis in the proximal tibiotarsus was covered in an extensive surface network of extracapsular retinacular vessels (ECRVs) (Figs 21 and 22). These ECRVs were the source of EVCs to the hyaline cartilage of the cranial epiphysis and PEVs to the underlying physis. On the craniomedial aspect of the proximal tibiotarsus there was a vessel which coursed around the cranial surface and anastomosed freely with the ECRVs (Fig 24). Branches from this vessel also directly penetrated the cartilaginous epiphysis to form EVCs.

The non load-bearing intra-articular surface of the cranial tibiotarsus was covered in a network of ICRVs. The ICRVs formed short EVCs to the underlying cartilaginous epiphysis but few PEVs.

A large intra-articular vessel (IAV) originated from the soft tissue of the lateral joint capsule between the fibula and the lateral cnemial crest. This vessel coursed intra-articularly to

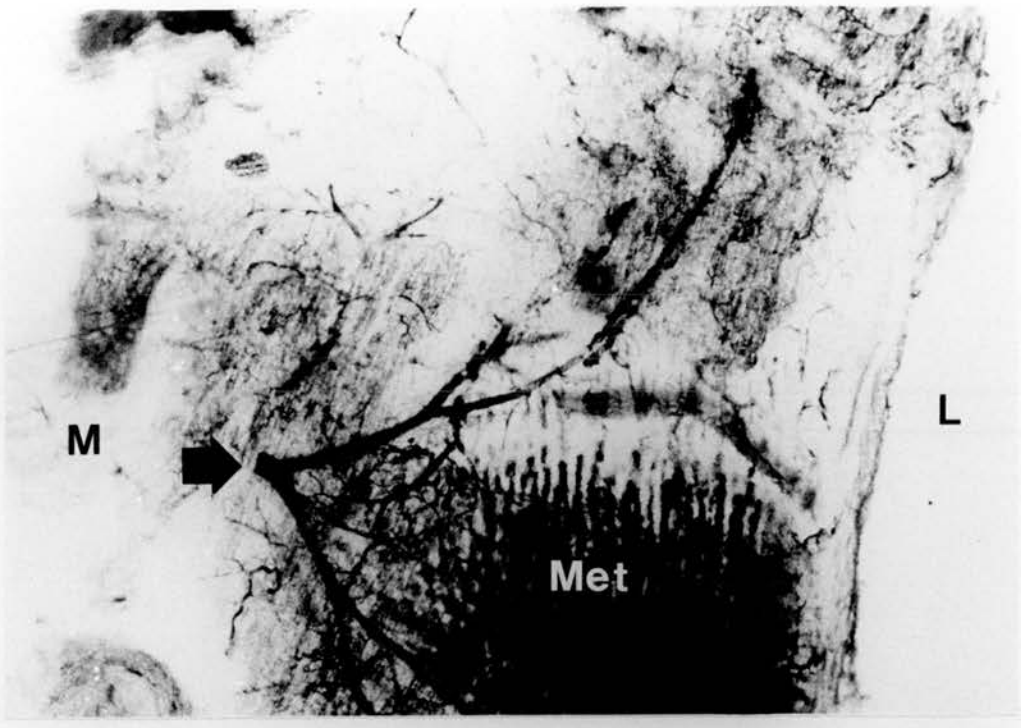


Fig 24. The cranial surface of the left proximal tibiotarsus from a 6 week old S line. The vessel (arrowed) originates from the medial crural artery and supplies the cranial ECRVs. 1mm slab x10.

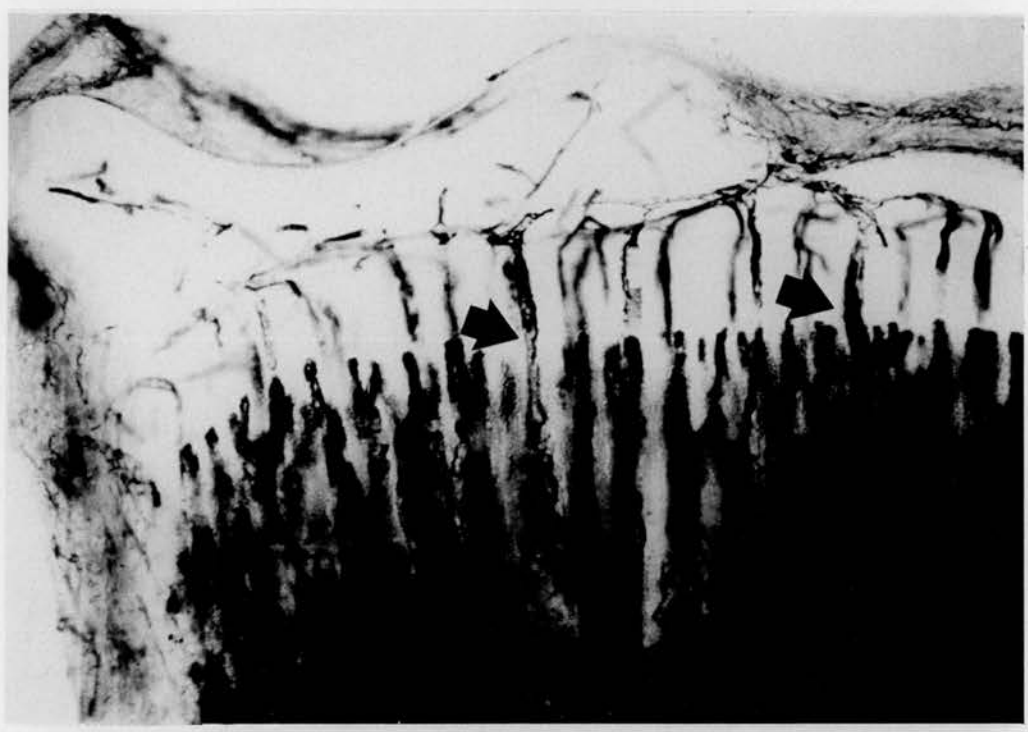


Fig 25. Coronal section of the proximal tibiotarsus from a 7 day old S line. Transphyseal PEVs (arrowed) anastomose with the newly formed irregular array of MVs. 1mm slab x40.

then enter the cartilaginous epiphysis on the craniolateral aspect of the intercondylar eminence (Fig 22 and 25). From this point EVCs radiated to supply the centre of the cartilaginous epiphysis and form PEVs to the underlying physis.

Formation of a fully functional growth plate.

In day old specimens MVs were only seen around the periphery of the metaphysis. Long transphyseal PEVs extended, through the physis, deep into the diaphyseal cone of cartilage (Fig 26). Most of the transphyseal PEVs were from EVCs which derived from the IAV.

By two days of age the MVs were branching towards the centre of the metaphysis, but long transphyseal PEVs were still apparent (Fig 27).

The dividing and branching MVs had formed a complete array across the metaphysis by five days of age. Frequently in birds between five and nine days of age there were transphyseal vessels, which originated as PEVs and anastomosed with MVs (Fig 25). Individual MVs were irregular in height and the arrays were unevenly spaced.

Sections from some of the young specimens were stained for calcium with silver using the von Kossa method. In the young specimens, where there was not a complete array of MVs, there was no calcification of the hypertrophic physeal cartilage.

A fine network of retinacular vessels was present over the articular surface of the condyles in day old specimens. These

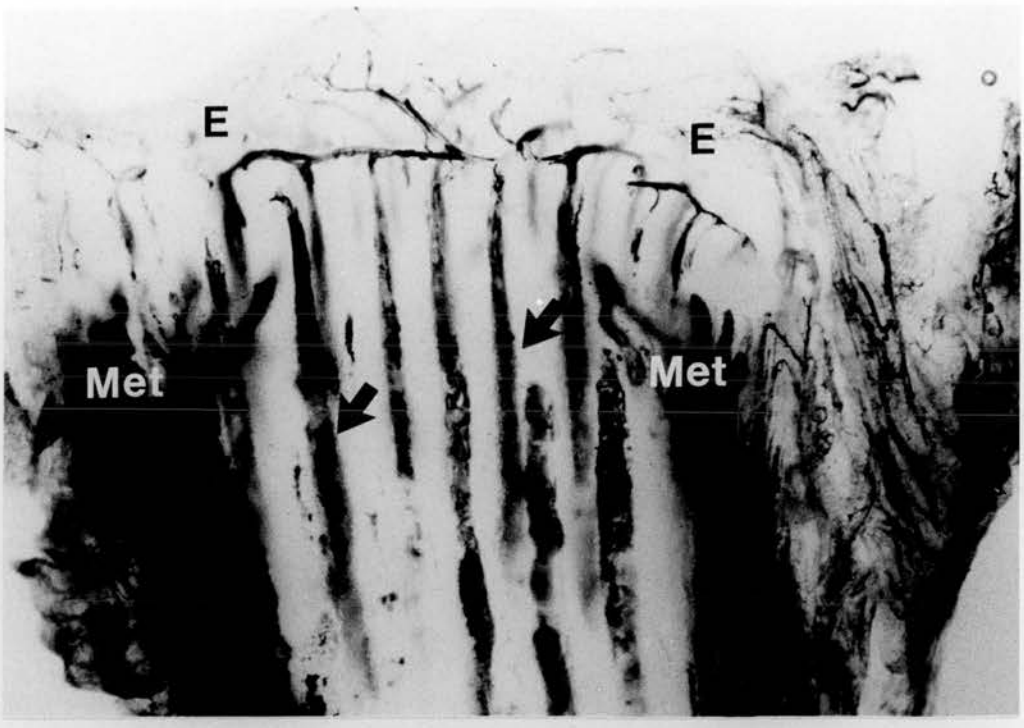


Fig 26. The proximal tibiotarsus from a day old S line. MVs are forming a collar around the periphery of the metaphysis. The cartilaginous metaphysis is penetrated by elongated transphyseal PEVs (arrowed). 1mm slab x25.

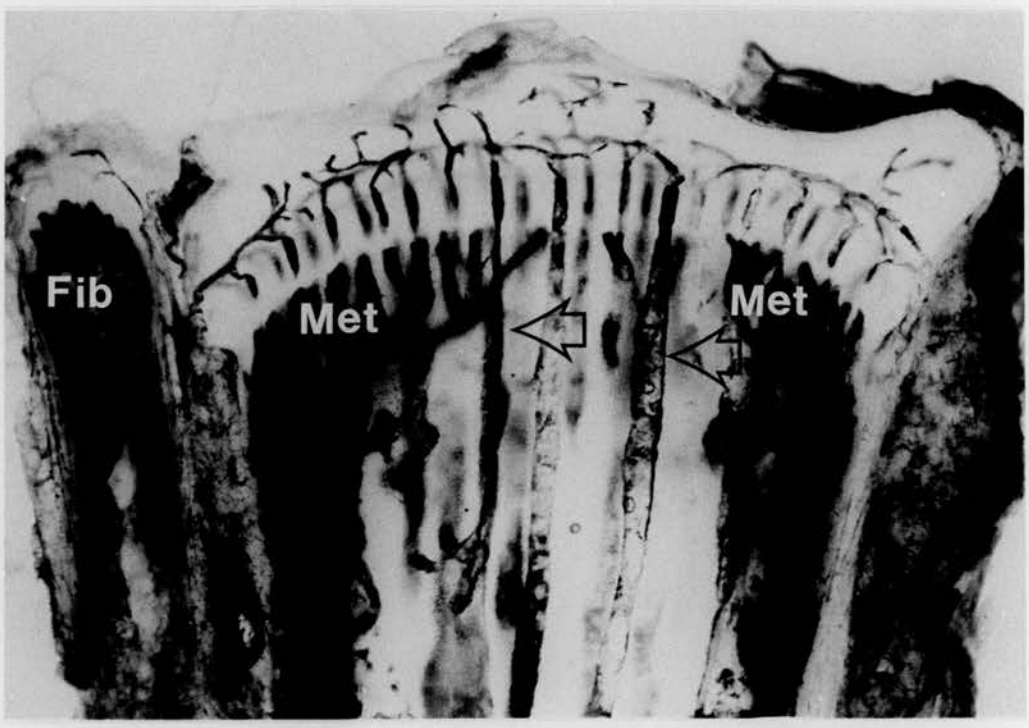


Fig 27. The proximal tibiotarsus of a 2 day old S line. The MVs are dividing to spread across the metaphysis. Only the centre of the metaphysis is penetrated by transphyseal PEVs (arrowed). 1mm slab x 25.

retinacular vessels had disappeared by two days of age. The large vessel on the cranial surface of the tibiotarsus, which supplied the ECRVs,was not apparent in specimens less than seven days of age.

Formation of the epiphyseal ossification centre (EOC).

In half of the six week old birds the EOC was present. By ten weeks of age the EOC was present in all of the specimens. The main supply of EVCs to the EOC originated from the ECRVs on the cranial surface of the tibiotarsus. There was also a vascular contribution to the EOC from the ICRVs on the proximal surface of the cranial tibiotarsus. Prior to formation of the EOC there was local hypertrophy of EVCs (Fig 28). Initially the EOC was spherical, centred just caudal to the cranial cnemial crest. The periphery of the centre was highly vascular with budding capillary loops slowly eroding into the surrounding epiphyseal hyaline cartilage. By fifteen weeks of age the EOC occupied most of the cranial cartilaginous epiphysis and cnemial crests (Fig 29). The EOC did not extend caudally into the condyles, but small tufts of vessels from the EOC penetrated the cranial periphery of the epiphyseal hyaline cartilage of the condyles.

At ten weeks of age the EVCs, which supplied the cranial physeal PEVs, coursed through the layer of hyaline cartilage between the ossified epiphysis and the physeal cartilage. These EVCs supplied the cranial physeal PEVs (Fig 24). There were three sources of EVCs to the cranial PEVs; cranial ECRVs,the IAV

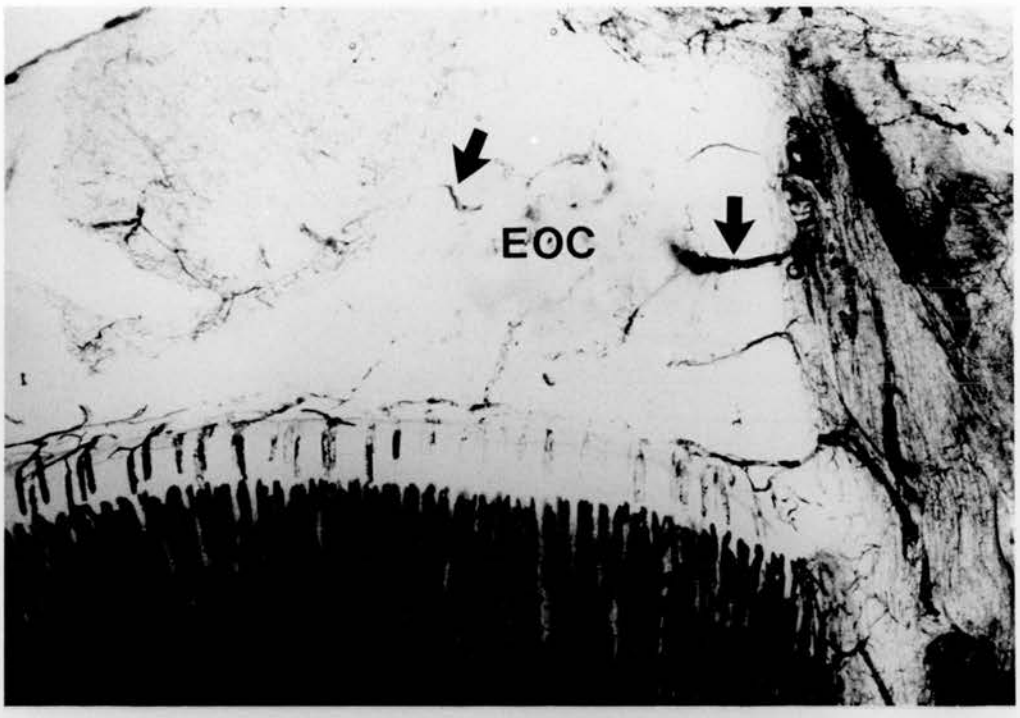


Fig 28. A sagittal section from the proximal tibiotarsus of a 6 week old S line. The EOC is starting to form in the highly vascular centre of the cnemial crest. The EOC is supplied by EVCs (arrowed) from ICRVs and ECRVs. 1mm slab x25.

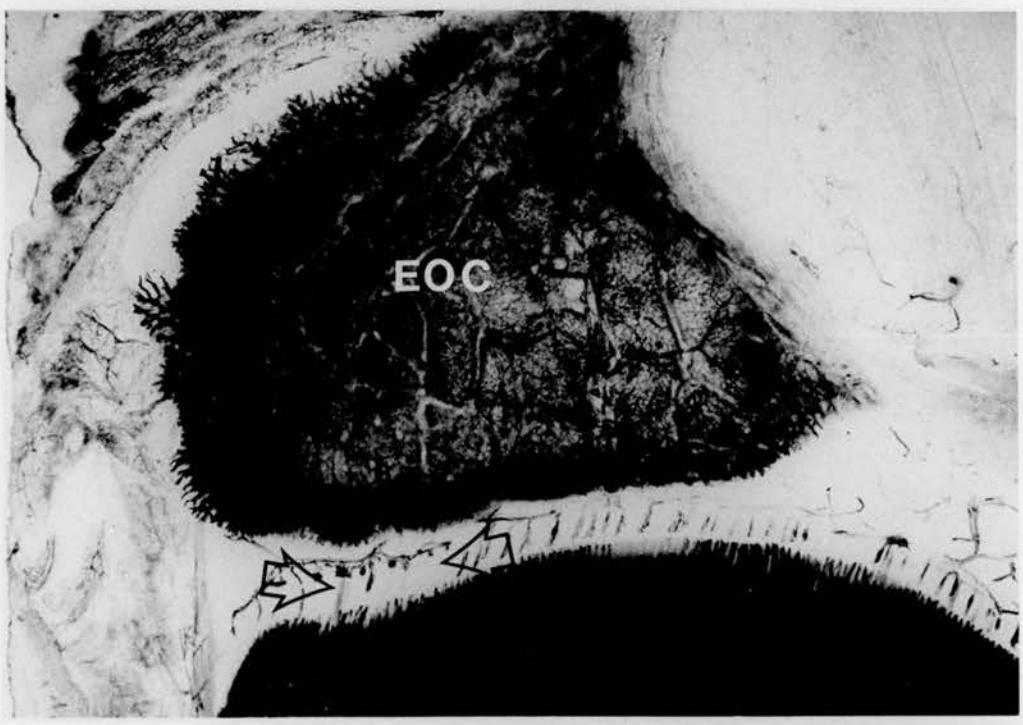


Fig 29. The proximal tibiotarsus from a 15 week old S line The EOC occupies the entire cnemial crest. PEVs in the cranial physis are occluded (arrowed). 1mm slab x10.

and,vessels which coursed through the EOC from the ICRVs on the surface of the proximal tibiotarsus. In some cases there were anastomoses between EVCs to the EOC and those EVCs which supplied PEVs (Fig 23). The general impression was that each EVC had a specific function of supplying either PEVs or the EOC.

In fifteen week old specimens the EOC had ceased to grow, and EVCs from the EOC supplied the underlying physeal cartilage with PEVs.

Cessation of growth.

At fifteen weeks of age the cranial epiphysis was fully occupied by the EOC. Closure of the physis was proceeded by a reduction in the number and size of PEVs. Bone union in the cranial segment of the proximal tibiotarsus, between the EOC and the metaphysis, had occurred by twenty weeks in females. This physis had only just started to close in males, of this age. As the hyaline cartilage of the caudal epiphysis became avascular,the physeal cartilage was being eroded by the advancing tufts of MVs. These MVs were ossifying the hyaline cartilage of the condyles in their wake.

The intra-articular vessel, penetrating the cartilaginous epiphysis on the craniolateral aspect of the intercondylar eminence, persisted as a blood supply to the menisci and cruciate ligaments.

Vascular defects occurring during growth.

Abnormalities occurred in four specimens. In a nine day old bird there was a metaphyseal defect due to delayed endochondral ossification of the physeal cartilage. The MVs inferior to the lesion were blunt ending and irregularly spaced (Fig 30). In a two week old bird there was a thickening of the physeal cartilage in the medial condyle. The PEVs in the area of cartilage thickening were enlarged and some bifurcated. In the proximal tibiotarsus from a ten week old bird, there was thickened physeal cartilage in the medial condyle. In the proximal tibiotarsus adjacent to the fibula of a number of specimens there was minor thickening of the physis in association with elongation of some of the PEVs. Occluded PEVs were in the cranial physis of the proximal tibiotarsus of a fifteen week old bird, between the EOC and metaphysis (Fig 29).

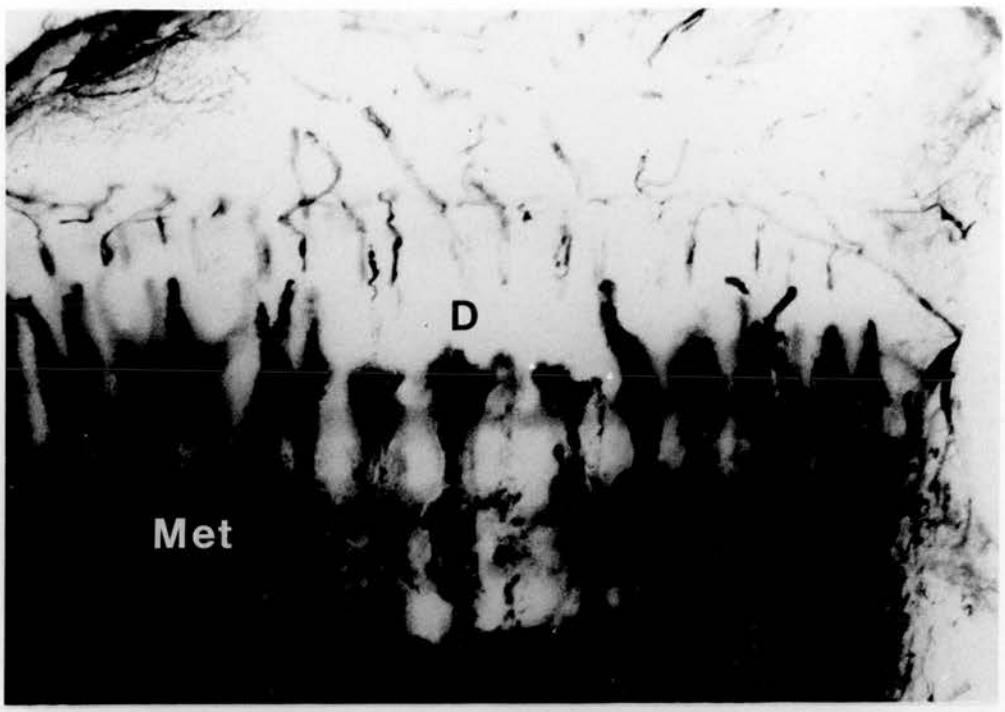


Fig 30. The proximal tibiotarsus from a 9 day old S line. There is disturbed MV penetration of the physeal cartilage (D). The MVs underlying the physeal defect are blunt ending and thickened. 1mm slab x40.

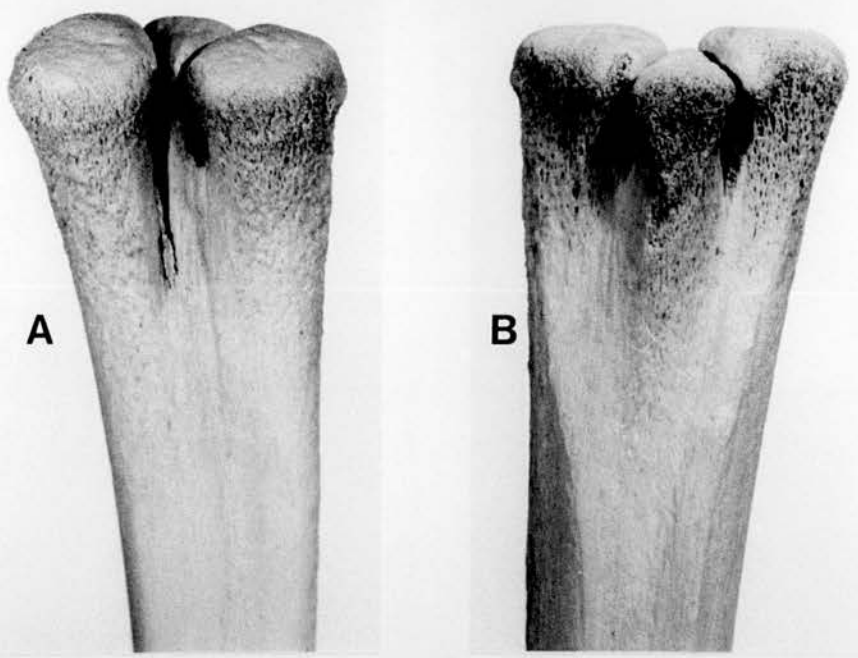
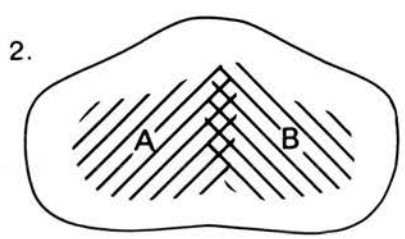
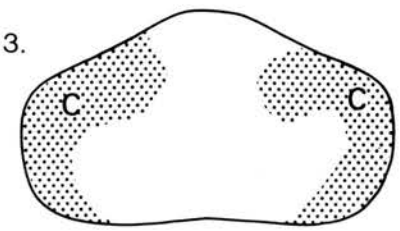
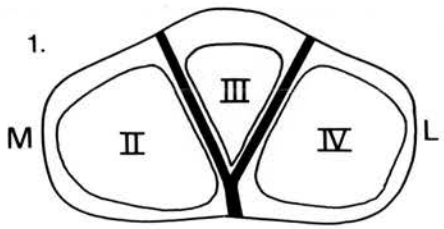
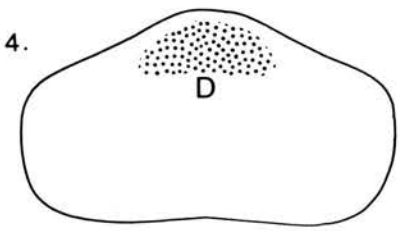


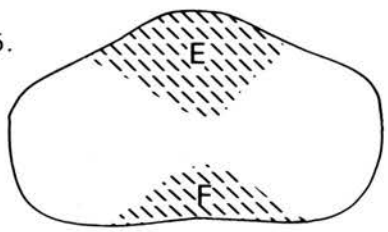
Fig 31. The cranial (A) and caudal (B) aspects of the bone extremities of the 3 synostosing metatarsal bones. The two vascular foramina subdivide the proximal metatarsus into 3 metaphysi which share a common cartilaginous epiphysis.

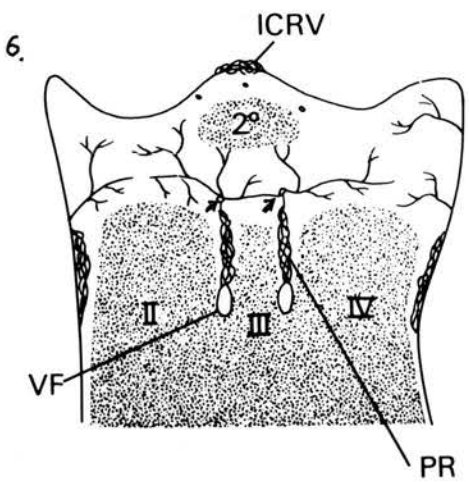

2.
A
B

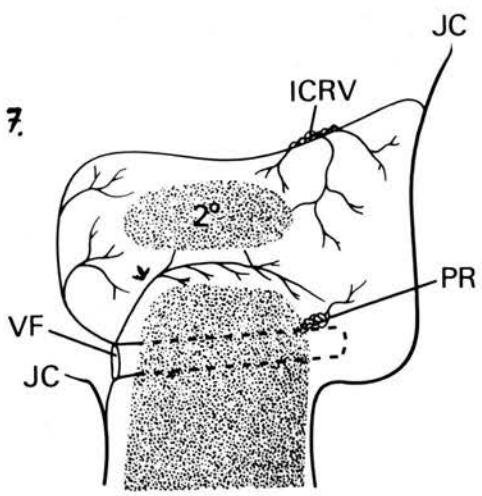

3.
C
C


1.
M
II
III
IV
L


4.
D


5.
E
F


6.
ICRV
2°
II
III
IV
VF
PR


7.
JC
ICRV
2°
PR
VF
JC

PROXIMAL TARSOMETATARSUS

In the first few days after hatching the vascular pattern of the developing tarsometatarsus became established. The length and complexity of the EVCs increased with growth. Throughout the growth period, however, individual EVC systems continued to supply the same sector of the cartilaginous epiphysis. The vascular supply in the proximal tarsometatarsus is diagrammatically represented in Fig 32. The description of the vascularity of the proximal tarsometatarsus is divided into:

i) Vascular pattern.
ii) Development of the vascular pattern.
iii) Epiphyseal ossification centre.
iv) Cessation of growth.

Vascular pattern.

The diaphysis of the tarsometatarsus consisted of three synostosing metatarsal bones, which separate proximally (Fig 31) and distally. The metatarsi each have an individual proximal physis, but share a common cartilaginous epiphysis. The epiphysis includes the hypotarsus on the plantar aspect of the proximal tarsometatarsus (Baumel,1979). Perichondrial vessels encircle the physis of each of the three proximal metatarsi. There were extensive anastomoses and intertwining of the perichondrial ring

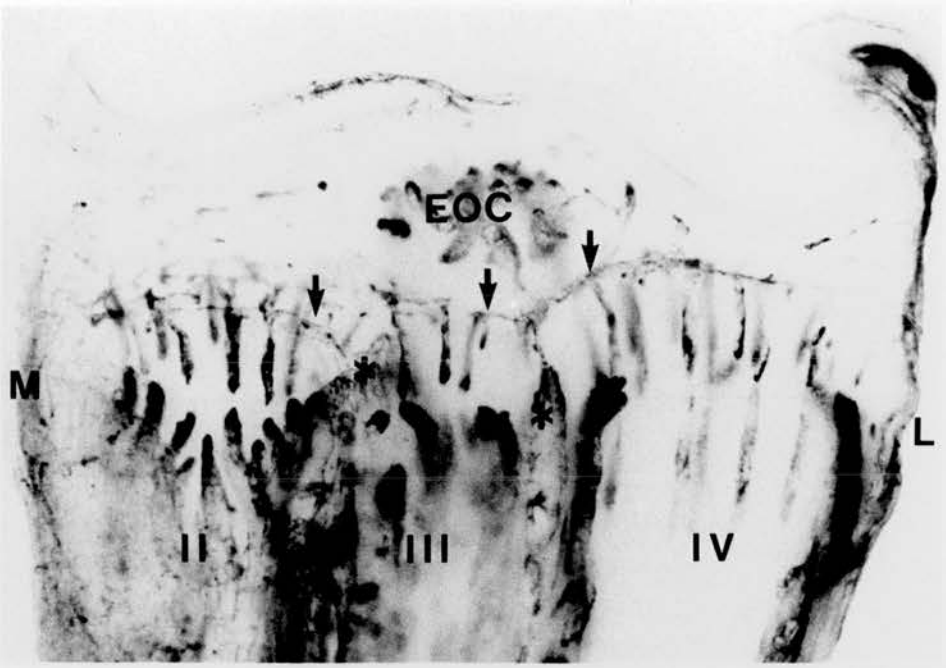


Fig 33. The proximal tarsometatarsus from a 2 day old S line. EVCs (arrowed) from the arcade of inter metatarsal perichondrial vessels (*) extend through the cartilaginous epiphysis. The 3 metatarsi (II, III and IV) all have MVs encircling the periphery of the metaphysis.The metatarsi are penetrated by transphyseal PEVs. 1mm slab x25.

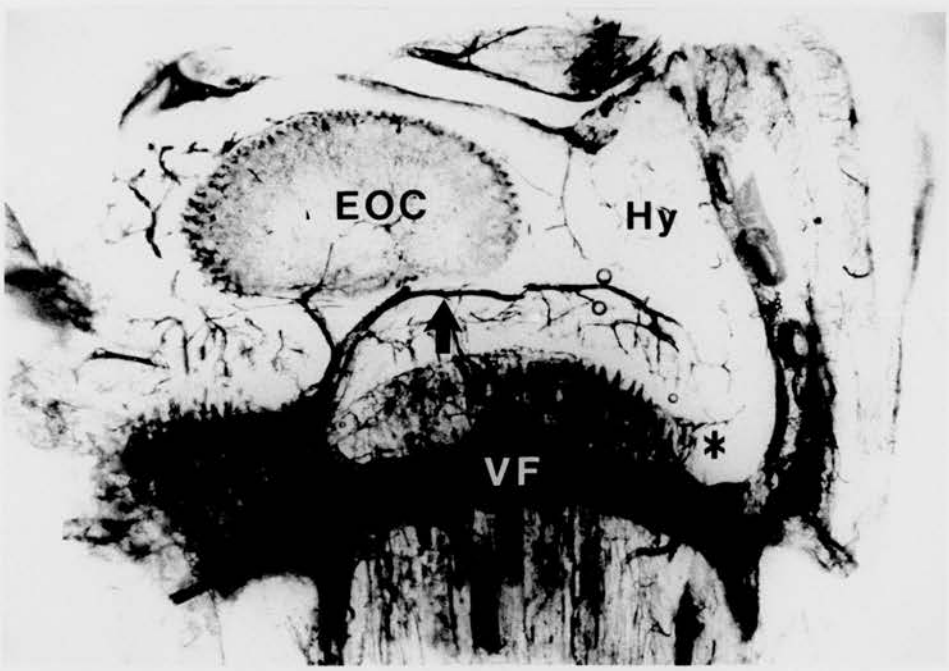


Fig 34. A sagittal section from the proximal tarsometatarsus of a 4 week old S line. The vascular foraman is situated below the principal EVC (arrowed). The hypotarsus is supplied by EVCs from ICRVs and the perichondrial vessels (*). 1mm slab x16.

vessels in the region of contact between the metatarsi (Fig 32 and 33).

The intermetatarsal perichondrial ring vessels formed EVCs and PEVs which supplied the surrounding epiphyseal hyaline cartilage. Perichondrial ring vessels on the plantar aspect of the metatarsi were the source of EVCs to the hypotarsus and the caudal periphery of the metatarsi (Fig 34). The rest of the perichondrial ring vessels although well developed did not form EVCs.

The plantar tarsal artery, a branch of the cranial tibial artery, divided on the dorsal surface of the proximal tarsometatarsus prior to traversing the medial and lateral proximal vascular foramina of the tarsometatarsus (Fig 32, 34 and 37). The two branches emerged from their respective foramina on the plantar medial and plantar lateral aspects of the hypotarsus. The medial foramen was situated between the second and third metatarsi below the perichondrial ring vessels. The lateral foramen similarly situated between the third and fourth metatarsi. The two branches of the plantar tarsal artery anastomosed with the perichondrial vessels along the length of the foramen. The depth of the vascular foramen relative to the physis increased with age.

The plantar tarsal artery, prior to dividing and entering the foramina, branched to form a number of vessels. One of these penetrated the cartilaginous epiphysis and promptly bifurcated to supply the two principal EVCs (Fig 34). The two principal EVCs each coursed caudally in the cartilaginous epiphysis proximal to the medial and lateral vascular foramina. The principal EVCs

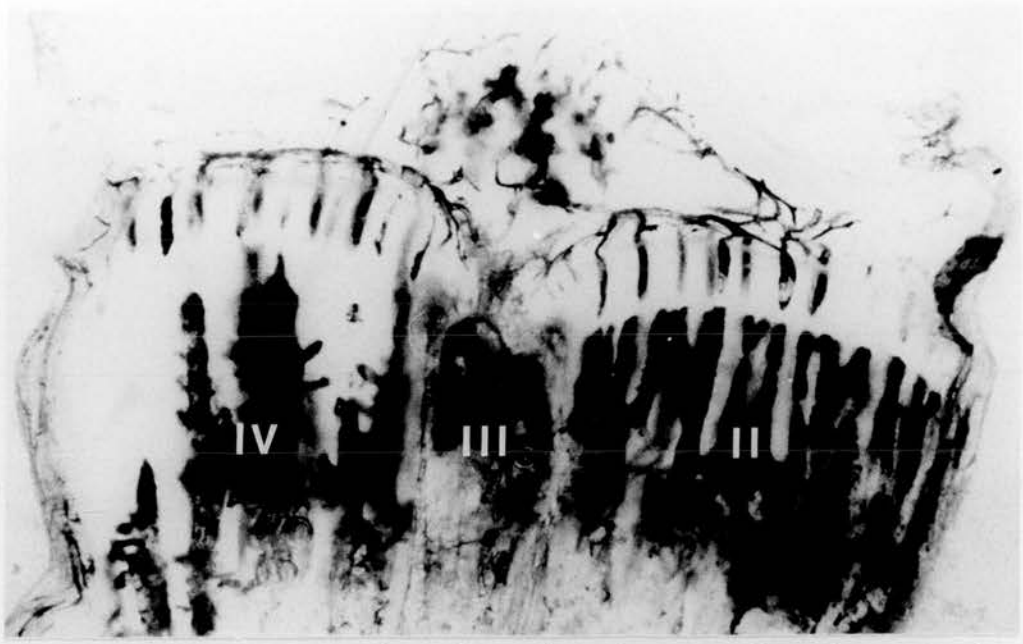


Fig 35. A 7 day old S line with disturbed MV invasion of the metaphysis in the IV metatarsal. The PEVs are normal. MVs are thickened, have lateral branches and fail to penetrate the retained cartilage. 1mm slab x25.

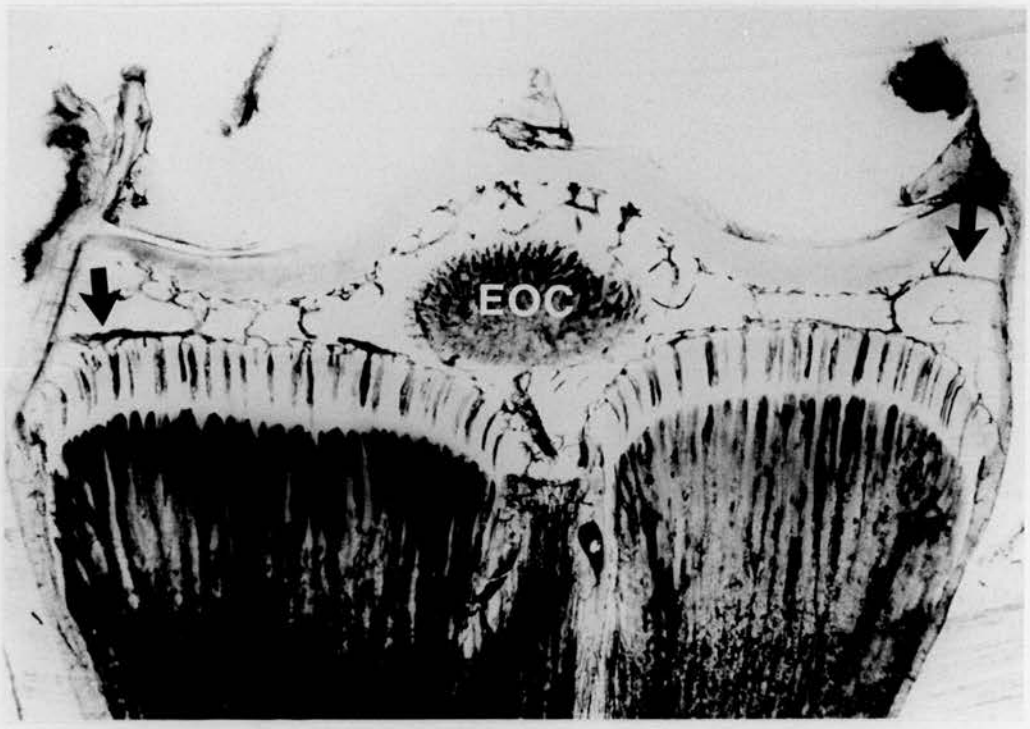


Fig 36. The proximal tarsometatarsus from a 3 week old S line. EVCs are forming a "halo" around the EOC. There are ECRVs on the medial and lateral aspects of the tarsometatarsus supplying EVCs (arrowed). 1mm slab x16.

anastomosed with the underlying perichondrial vessels.

The two principal EVCs arborised extensively, forming a network of EVCs across much of the cartilaginous epiphysis supplying the underlying physeal cartilage with PEVs. Branches from the principal EVCs extended caudally into the cartilaginous hypotarsus. The developing EOC was also vascularized by branches from the principal EVCs (Fig 34). The medial principal EVC was of greater diameter than the lateral.

The other small branches from the the plantar tarsal artery, which left the artery near its point of entry to the vascular foramen, were responsible for a network of retinacular vessels. These retinacular vessels extended from the cranial surface of the cartilaginous epiphysis onto the medial and lateral surfaces of the proximal tarsometatarsus. The cranial ECRVs continued through the joint capsule to become ICRVs which then supplied the periphery of the cranial cartilaginous epiphysis with EVCs and PEVs.

The plantar tarsal arteries, after emerging from their vascular foramina, were the source of ECRVs to the caudomedial and caudolateral surfaces of the hypotarsus. On the medial and lateral surfaces of the cartilaginous epiphysis there were networks of ECRVs which supplied EVCs to the periphery of the condyles (Fig 36). The ECRVs formed short EVCs to the local hypotarsal and epiphyseal hyaline cartilage. Also a few PEVs were formed in the peripheral physeal cartilage. Small branches from the plantar tarsal arteries were the source of ICRVs on the proximal surface of the hypotarsus which extended onto the intercondylar cartilage of the cartilaginous epiphysis. These

ICRVs supplied the underlying hypotarsal and epiphyseal hyaline cartilage with EVCs (Fig 34).

The EVCs of the proximal tarsometatarus which supplied the physeal PEVs, lay in one plane which was parallel with the physis. PEVs descended vertically from this network of EVCs to penetrate the physis. There were also EVCs which arose from this network to supply the rest of the cartilaginous epiphysis. The thicker cartilage in the periphery of the condyles contained more extensive EVC systems than the thinner epiphyseal hyaline cartilage of the cotyles.

There were anastomosing connections amongst the EVCs of the epiphyseal network. This interconnecting of EVCs produced a lattice like appearance to the vascular canals in the cartilaginous epiphysis above each metatarsal physis. Connections were seen to occur between vessels from the same and also from different EVCs. There was a greater number of blind ending "glomerulus" like EVCs in the thicker cartilage of the cartilaginous epiphysis.

Development of the vascular pattern.

Each of the three proximal metaphyses in the day old metatarsi contained an extensive cone of cartilage (Fig 33). Elongated transphyseal PEVs extended deeply into the cartilage cores. MVs encircled the periphery of each metaphysis. The majority of EVCs originated from the intermetatarsal perichondrial ring. The two principal EVCs by two days of age had become the

predominant vascular supply. MVs now formed a complete array across the metaphysis of the third metatarsal (III); but the IInd and IVth metatarsals still had incomplete MV arrays and transphyseal PEVs. At five days of age MV arrays were still incomplete across the metaphyses of half of the metatarsi examined.

At seven days of age in 50% of the proximal tarsometatarsi there was delayed MV invasion of either the medial or lateral metaphysis (II or IV). MV invasion was considered to be delayed because the PEVs were no longer transphyseal and a completed MV array was not present across the metaphysis. The avascular cartilage, which occupied the metaphysis, was being eroded by enlarged blunt ending MVs (Fig 35). The MVs as they encircled the cone of "retained" cartilage sent small branches into it. By nine days of age a very small avascular cone was occasionally present in the centre of either the medial or lateral metaphysis. The MV arrays were all normal in the metataphyses of the fourteen day old birds.

Epiphyseal ossification centre (EOC).

The EOC was present in all day old birds as a small spherical conglomerate of EVCs found in the hyaline cartilage of the intercotylar epiphysis (Fig 33). A well developed vascular supply from the two principal EVCs developed to the EOC in the first fortnight after hatching. The EOC then expanded rapidly and advanced into the neighbouring hyaline cartilage. Surrounding the

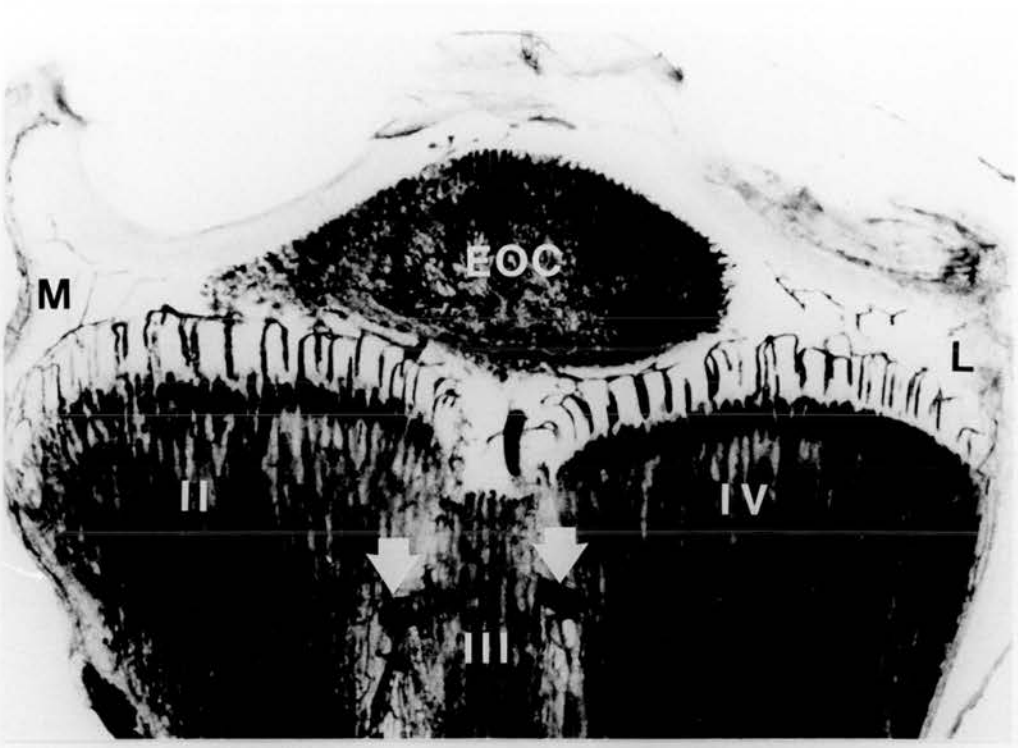


Fig 37. The proximal tarsometatarsus from a 6 week old S line. Th EOC is extending into the epiphysis of the medial condyle. The 2 vascular foramina are arrowed. 1mm slab x10.

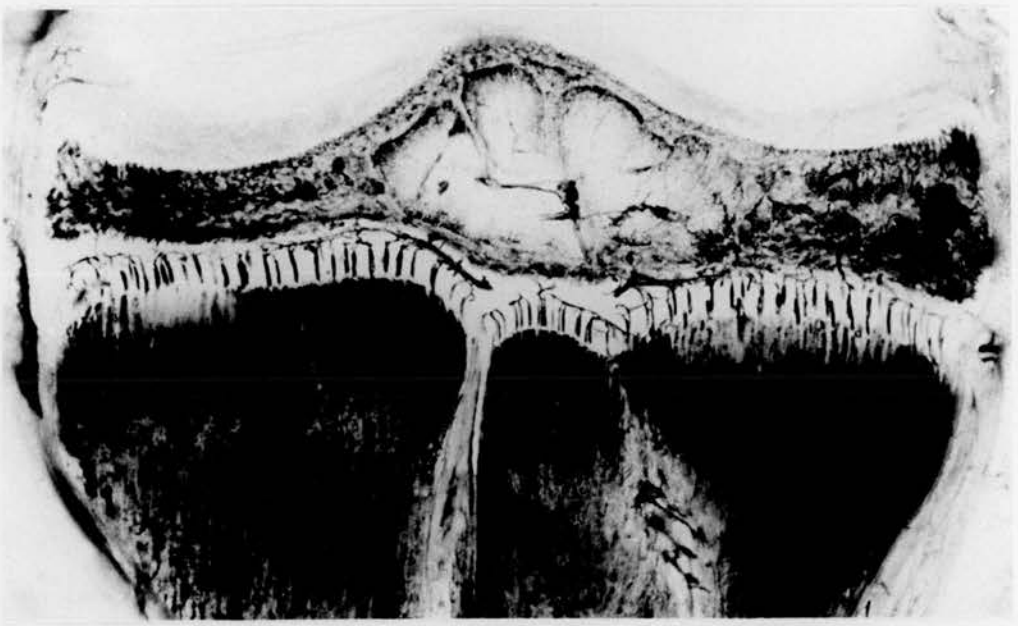


Fig 38. The proximal tarsometatarsus from a 10 week old S line. The EOC occupies the entire epiphysis, apart from a narrowed layer of hyaline cartilage between the ossified epiphysis and the physis. 1mm slab x10

EOC there was a "halo" of EVCs all equidistant from its periphery (Fig 36). The layer of cartilage between the articular cartilage and the EOC became reduced in thickness as the EOC expanded. The expanding EOC then overran the "halo" of EVCs. By forty two days the EOC had enlarged so as to lie just below the intercotylar surface of the hock joint. The EOC had now extended into the cartilaginous epiphysis of the medial cotyle, but was not yet invading the lateral cotyle (Fig 37). ICRVs on the cranial cartilaginous epiphysis and from the intercotylar surface now augmented the vascular supply of the EOC.

The rate of epiphyseal ossification was such that by seventy days of age most of the cartilaginous epiphysis (Fig 38) and hypotarsus had been ossified by the enlarging EOC. There was now only a narrow band of hyaline cartilage between the physis and ossified epiphysis. This stratum of hyaline cartilage carried the EVCs which supplied the PEVs. These EVCs originated from the same source as in younger birds, but there were frequent vascular connections with the vessels of the EOC. The EVCs derived from perichondrial vessels now only formed short EVCs and few PEVs. Virtually no epiphyseal hyaline cartilage remained in birds of fifteen weeks of age, and the PEVs originated from the ossified epiphysis (Fig 39).

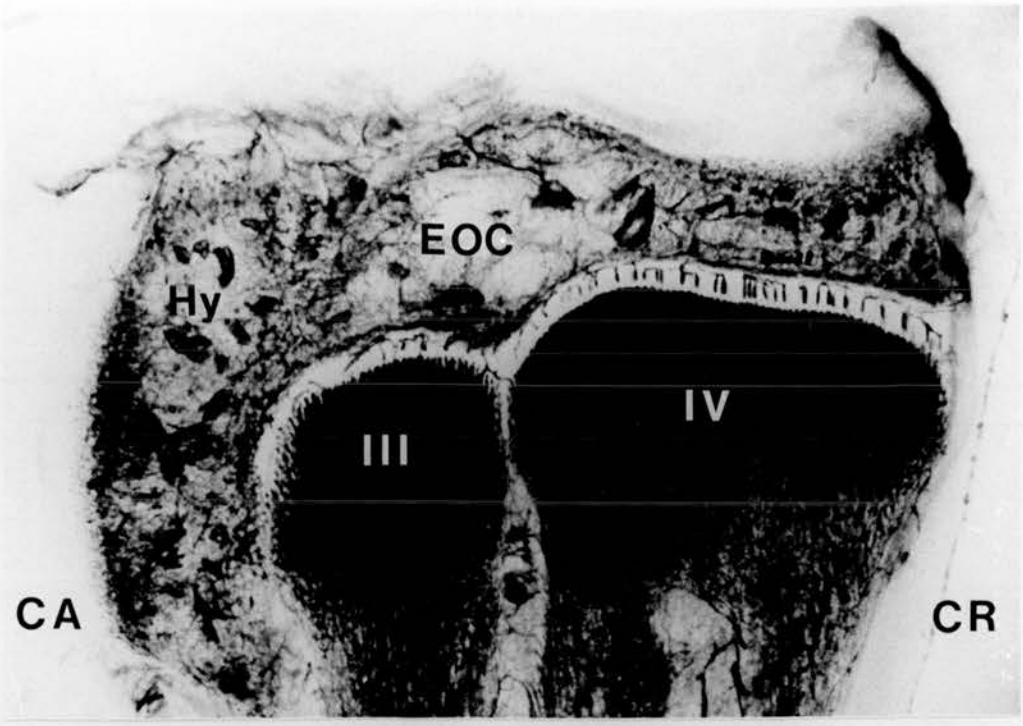


Fig 39. A sagittal section of the proximal tarsometatarsus from a 15 week old S line. All the hyaline cartilage in the epiphysis and hypotarsus has ossified. 1mm slab x6.

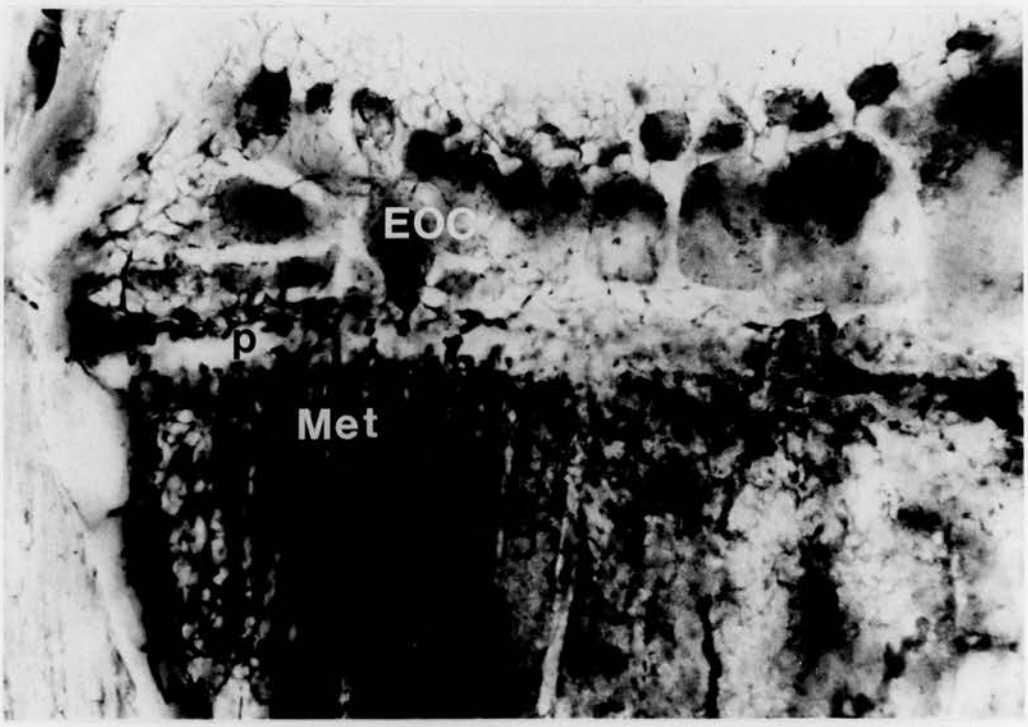


Fig 40. The proximal tarsometatarsus from a 20 week old S line. The MV from the IVth metatarsal are crossing the physis to become incorporated within the ossified epiphysis. 1mm slab x16.

Cessation of growth

The number and size of PEVs, was reduced as the rate of growth slowed. Complete bone union between the ossified epiphysis and metaphysis had occurred in all the female birds by twenty weeks of age. In the male birds the physis was still undergoing closure at this age (Fig 40).

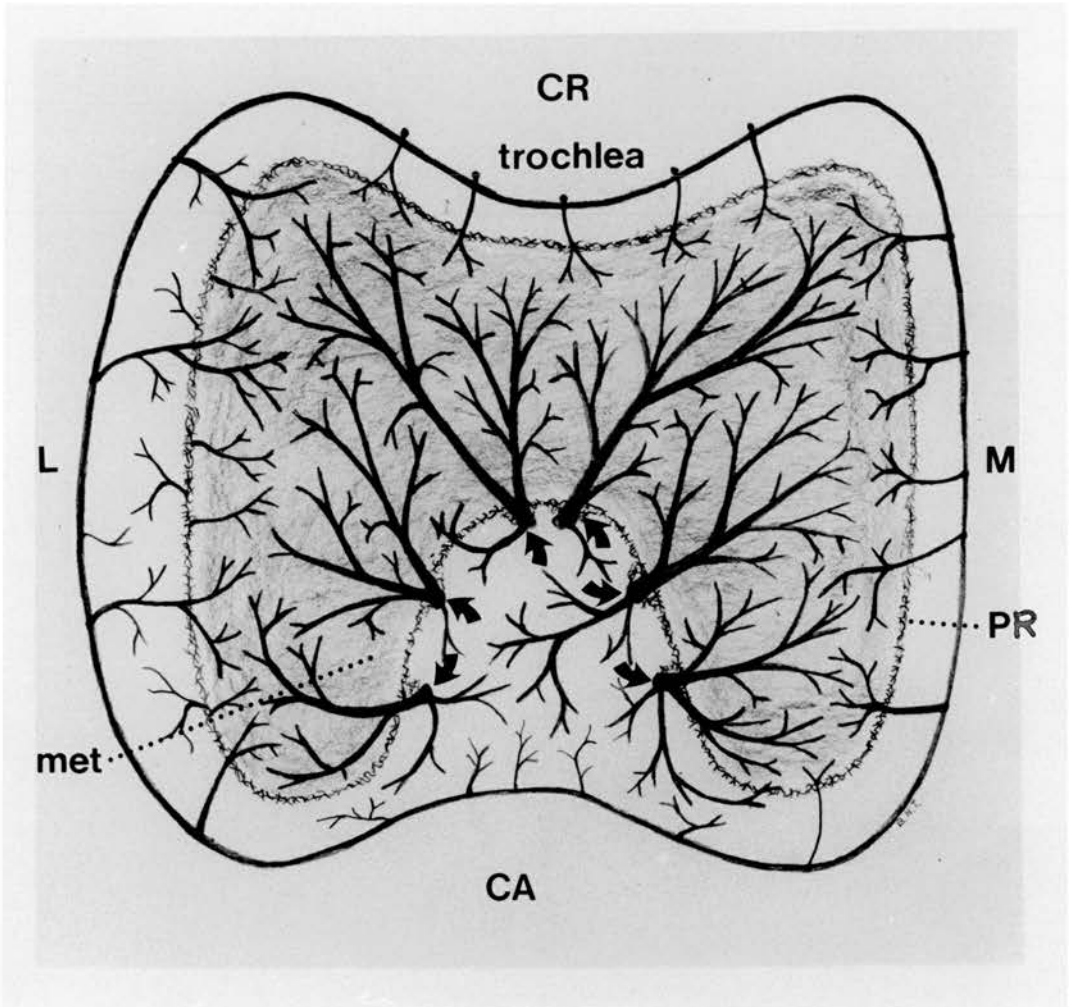


Fig 41. Diagrammatic representation of the EVCs in the cartilaginous epiphysis of the distal femur. There are six principal EVCs (arrowed) originating from a "horseshoe" of vessels in the caudal intercondylar region. The six principal EVCs divaricate through the cartilaginous epiphysis to form PEVs to the underlying physis. ICRVs on the the trochlea and the medial and lateral surfaces of the distal femur give rise to EVCs. The lateral perichondrial ring EVCs also supply EVCs.
L: lateral; M: medial and met: metaphysis.

DISTAL FEMUR

The vascular pattern of the developing distal femur was established in the first few days after hatching. There were however a number of modifications during growth to the general pattern. There was no EOC.

General pattern

The metaphysis of the distal femur was U-shaped when cut in transverse section (Fig 41). A ring of perichondrial vessels encircled the physis. The caudal intercondylar and lateral epicondylar regions were both extensions of the cartilaginous epiphysis. There were ICRVs on the surface of the medial and lateral epicondyles.

A branch of the medial femoral artery, arising from near the medial epicondyle, continued across the cranial surface of the distal femur from where it divaricated over the surface of the trochlea (Fig 42). These branches gave rise to a network of retinacular vessels which extended caudally between the condyles of the distal femur and contributed to the vascular supply of the cruciate ligaments. The retinacular vessels of the trochlea were also a source of EVCs to the cranial periphery of the cartilaginous epiphysis, but these EVCs were not very extensive.

A branch of the popliteal artery on the caudal aspect of the distal femur penetrated the proximal aspect of the intercondylar

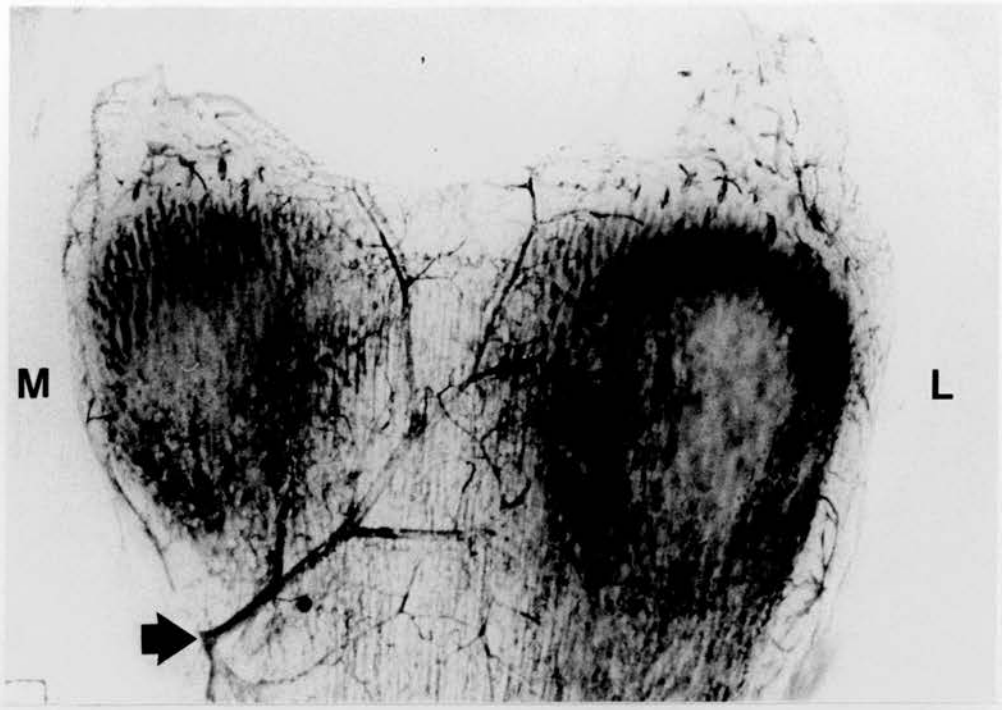


Fig 42. The distal femur of a 4 week old S line. The vessel (arrowed) extends from the medial condyle to divaricate across the surface of the trochlea. 1mm slab x16.

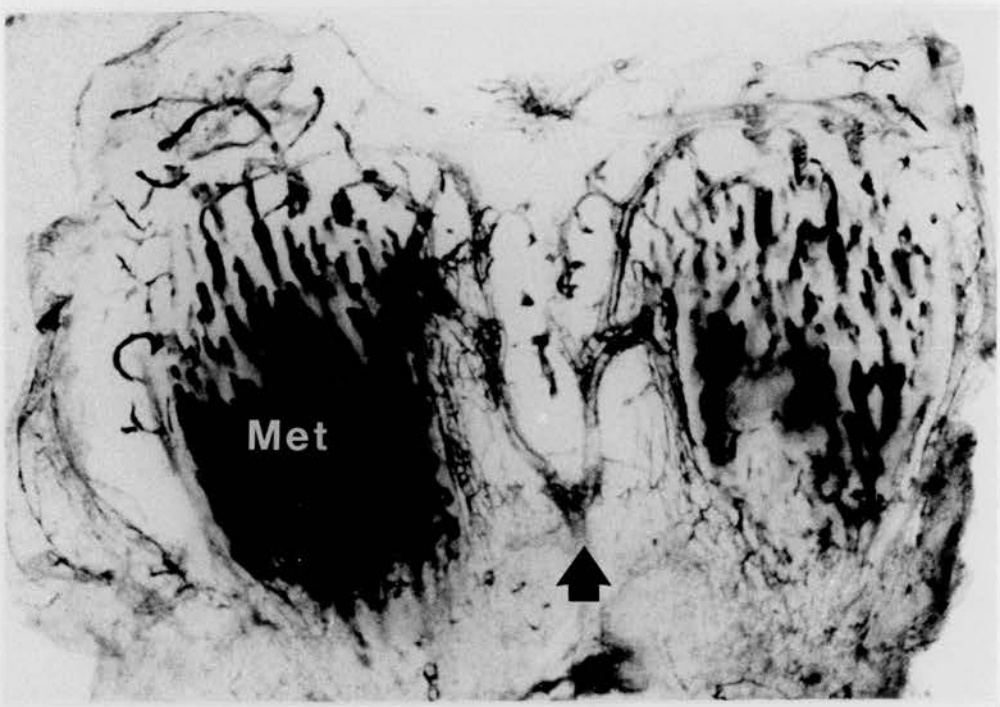


Fig 43. A section from the caudal aspect of the distal femur of a 4 week old S line. A branch (arrowed) from the popliteal artery subdivides in the caudal intercondylar cartilage to form the "horseshoe" of principal EVCs. 1mm slab x16.

cartilage (Fig 43). This vessel then radiated to form a crescent or horseshoe of six principal EVCs which were the main vascular supply to the cartilaginous epiphysis. The horseshoe of radiating EVCs was situated in the U-shape created by the caudal aspect of the metaphysis. The horseshoe of EVCs was adjacent to the perichondrial vessels of the caudal physis. There were frequent anastomoses between the principal EVCs and the adjacent perichondrial vessels. Retinacular vessels formed EVCs which supplied the periphery of the cartilaginous epiphysis of the condyles and also supplied PEVs to the underlying physis. The perichondrial ring vessels formed few EVCs, except in the lateral condyle. EVCs derived from the perichondrial ring made a significant vascular contribution to the epiphyseal hyaline cartilage of the lateral epicondyle and fibular trochlea (Fig 44 and 45).

The epiphyseal hyaline cartilage of the condyles was thicker and contained many more blind ending EVCs, than the thinner intercondylar cartilage. No vascular connections were seen to occur between EVCs in the distal femur.

Changes with age

Day old.

The metaphysis contained a cone of cartilage which was encircled by MVs progressing towards the physis. Transphyseal PEVs penetrated deeply into the cartilage core (Fig 46). In the metaphysis of the caudal condyles MVs were already arranged as

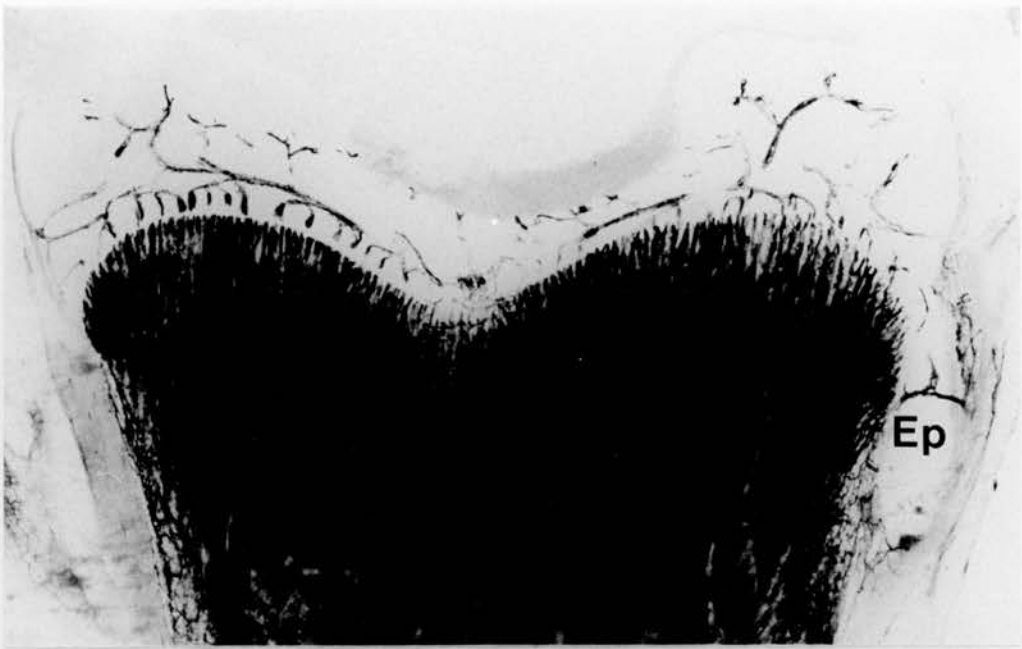


Fig 44. The distal femur from a 10 week old S line. The extensive cartilage in the lateral epicondyle is supplied by EVCs from the perichondrial vessels and ECRVs. 1mm slab x10.

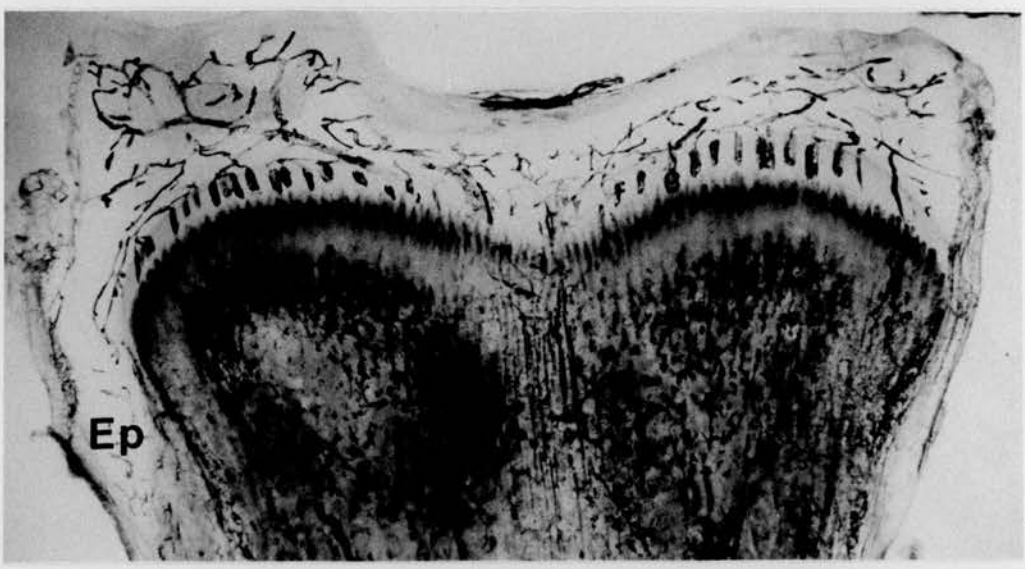


Fig 45. The distal femur from a 4 week old S line. The cartilaginous epiphysis of the condyles contains many blind ending EVCs. 1mm slab x16.

arrays and the six principal EVCs were the predominant vascular supply to the epiphyseal hyaline cartilage. Retinacular vessels only formed short, poorly developed EVCs.

Day two.

The arrays of MVs, which were now across all of the metaphyses, overlay the small remaining cartilage core (Fig 47). EVCs from the medial and lateral ICRVs were more extensive and supplied the peripheral physeal cartilage with PEVs. The lateral perichondrial ring now formed EVCs which by nine days of age supplied the cartilage of the lateral physis with PEVs.

Day 21.

The EVCs from the lateral perichondrial ring now supplied the outer third of the physeal cartilage in the lateral condyle with PEVs. The ICRVs on the lateral surface of the distal femur formed EVCs to the underlying epiphyseal hyaline cartilage, and the medial ICRVs functioned in a similar fashion. A few EVCs were derived from the vessels of the medial perichondrial ring. The ICRVs in the trochlea formed a lattice like network on the surface of the articular cartilage.

Day 28.

EVCs derived from the medial ICRVs now extended into the outer third of the epiphyseal hyaline cartilage of the medial condyle, and supplied PEVs to the underlying physis. EVCs from the lateral ICRVs were also more extensive than they had been in

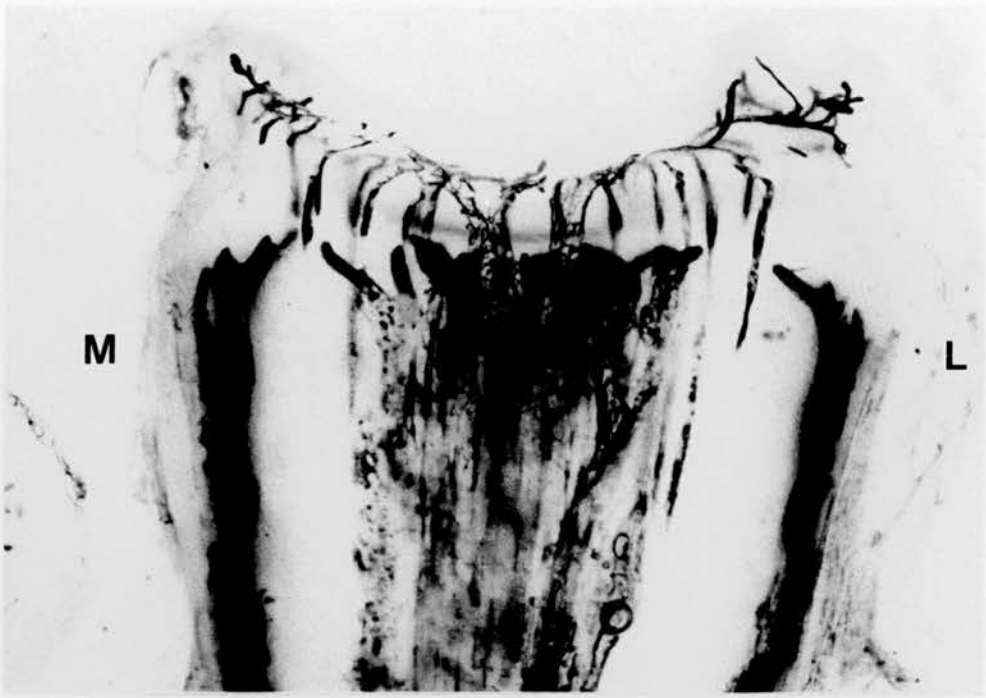


Fig 46. The distal femur of a day old S line. The metaphysis has a peripheral collar of MVs and the cartilaginous centre is penetrated by elongated transphyseal PEVs. 1mm slab x25.

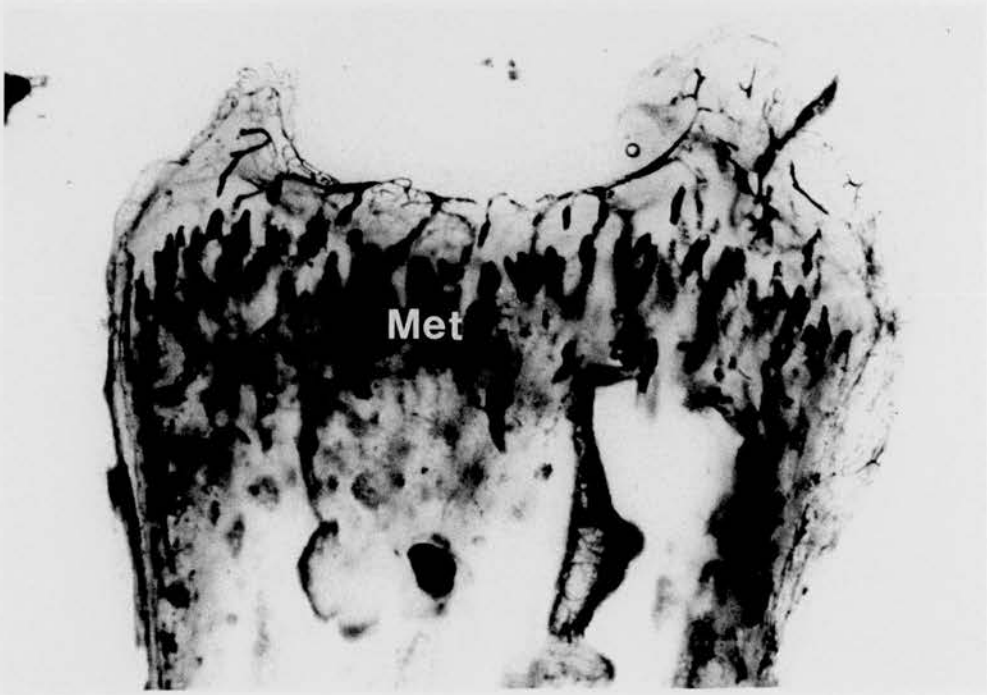


Fig 47. The distal femur from a 2 day old S line. The MVs now form an uneven array across the metaphysis. 1mm slab x25.

younger birds.

Day 28 to 105.

There was no marked change in the vascularity of the distal femur over this period. The EVCs became more expansive as the cartilaginous epiphyses grew in size.

Day 105.

In females the physeal cartilage was being overrun by the metaphysis. There were fewer perichondrial vessels and much of the epiphyseal hyaline cartilage was virtually avascular due to EVC occlusion (Fig 48). The invasion of MVs into the maturing physis was uneven, but was further advanced at the site of patent EVCs or PEV remnants (Fig 49). In the cartilage of the lateral epicondyle there was a dearth of patent EVCs.

There was an overall reduction in vascularity of the cartilaginous epiphyses in males, due to a wider spacing between EVCs and no increase in branching. The physis was reduced in width, PEVs were shorter, and there was an increase in the spacing between PEVs.

Complete ossification of the epiphyses had occurred by twenty weeks in the females but was still an ongoing process in males.

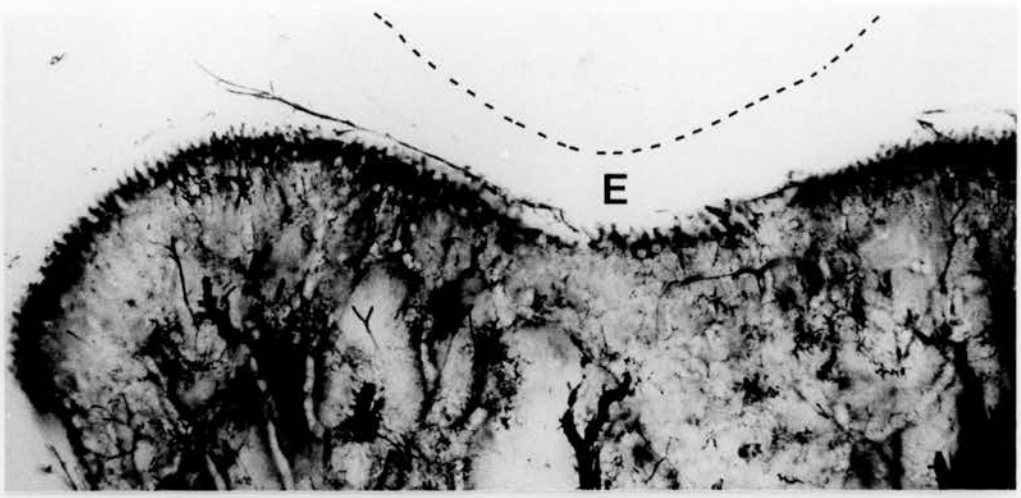


Fig 48. The distal femur from a 15 week old S line. The EVCs and PEVs are reduced in number and the latter are widely spaced and shortened. 1mm slab x10.

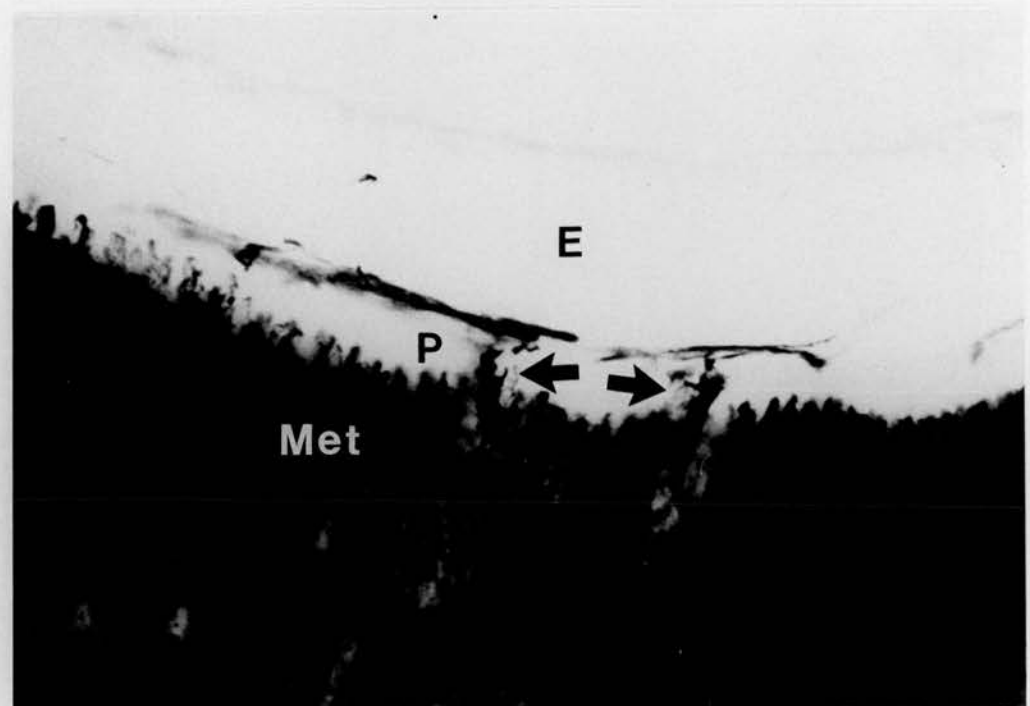


Fig 49. The distal femur from a 15 week old S line. The MVs are starting to cross the physeal cartilage. Two enlarged transphyseal PEVs (arrowed) are present which anastomose with the MVs. Where EVCs are present MV invasion is more rapid. 1mm slab x20.

DISTAL TIBIOTARSUS

The vascularity of the distal tibiotarsus followed a general pattern. That pattern was modified:

i) In the very young bird.

ii) By the development of the epiphyseal ossification centre.

iii) By physeal closure.

General pattern.

The epiphyseal hyaline cartilage of the distal tibiotarsus contained three centres of secondary ossification. These included the lateral and medial condylar centre (Fig 50 and 52). The third EOC was located in hyaline cartilage cranially, adjacent to the metaphysis and between the condyles at the point of attachment of the Pons Supratendineus (Fig 52). The physis was encircled by a perichondrial ring of vessels, which formed a fibro-vascular barrier between the third ossification centre and the metaphysis of the distal tibiotarsus (Fig 51). The third EOC was present in all the specimens examined.

A branch of the cranial tibial artery entered the epiphyseal hyaline cartilage in the cranial intercondylar region (Incisura Intercondylaris) lateral to the Pons Supratendineus (Fig 52). This was the main vascular supply to the cartilaginous epiphysis. This vessel divided after entering the cartilage to form EVCs.

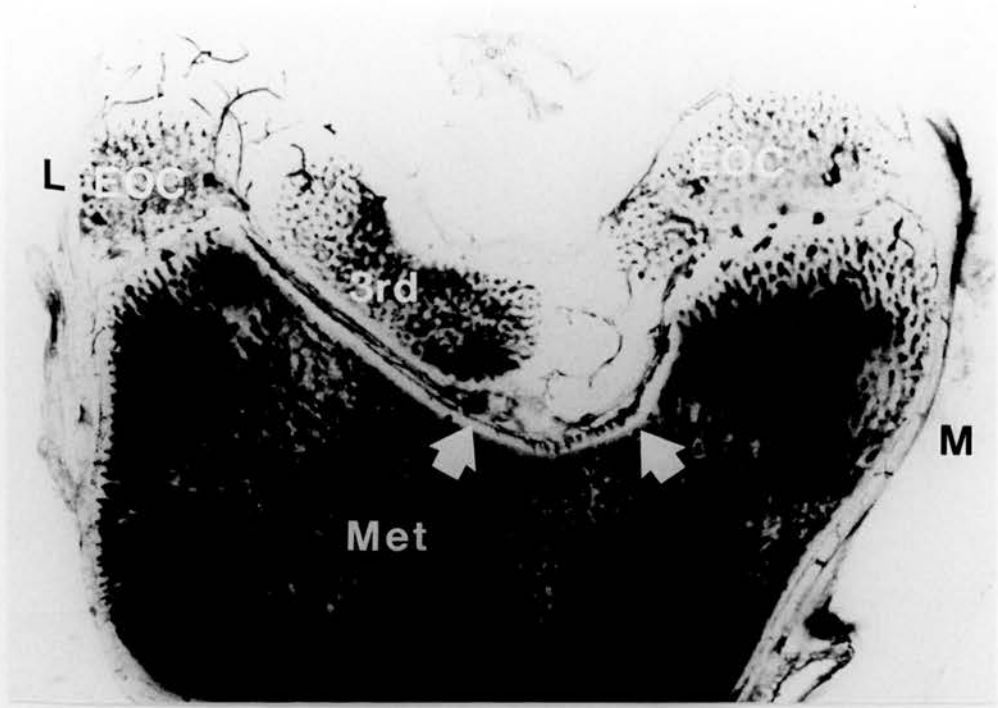


Fig 50. The distal tibiotarsus from a 4 week old S line. The paired medial and lateral EOCs and the third EOC are sectioned. The vascular crescent (arrowed) of perichondrial vessels supplies EVCs to all 3 EOCs. 1mm slab x10.

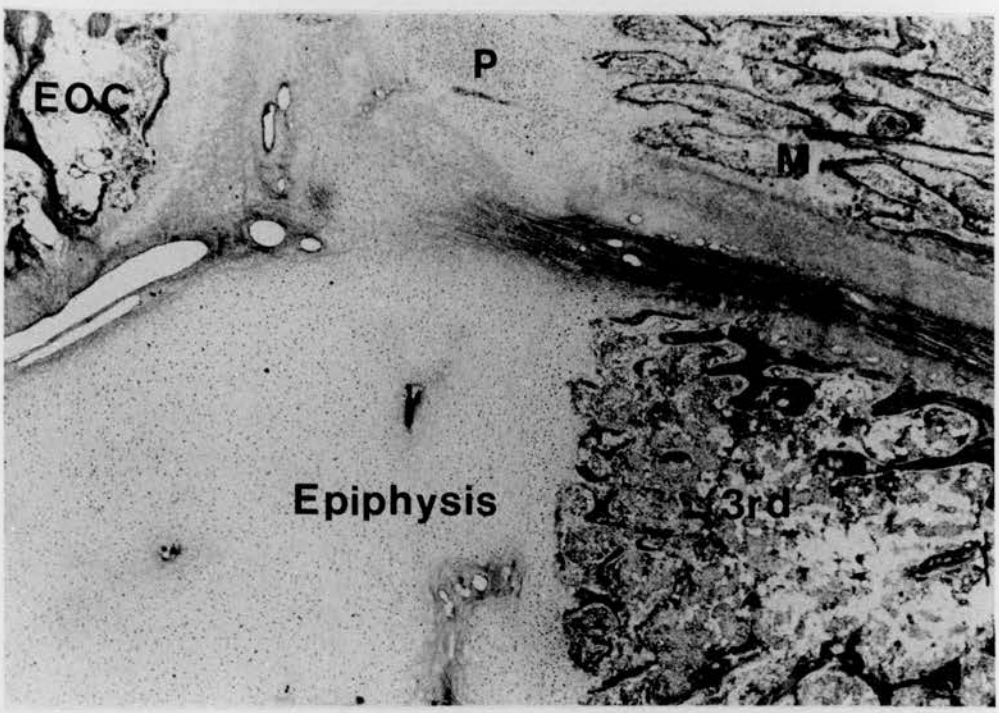


Fig 51. A histological section prepared from the slab shown in fig 50. The third EOC is separated from the metaphysis (M) by fibrovascular tissue. The lateral EOC is present in the top left of the photomicrograph. MGT x25.

some of which supplied the surrounding cartilage and third ossification centre. The main vessel then ramified to form a crescent of vessel, to the perichondrial ring between the condyles of the cranial physis. There were numerous vascular connections between this crescent and the adjacent vessels of the perichondrial ring. The crescent then gave rise to between five and seven principal EVCs. which ran axially through the cartilaginous epiphysis (Fig 53). Where the principal EVCs crossed the cranial margin of the physis there were further frequent anastomoses with the perichondrial ring. As they traversed the cartilaginous epiphysis the principal EVCs divided (Fig 54) to supply branches to the overlying epiphyseal hyaline cartilage and the paired condylar ossification centres. The principal EVCs were also the source of EVCs which subsequently formed PEVs.

ICRVs on the medial and lateral surface of the cartilaginous epiphysis formed EVCs. These EVCs which extended into the epiphyseal hyaline cartilage of the condyles. also supplied the paired condylar ossification centres (Fig 55). The EVCs from the medial ICRVs were more extensive than those from the lateral ICRVs.

The caudal tibial artery gave rise to a branch which, as it crossed the caudal surface of the distal tibiotarsus, produced numerous offshoots (Fig 56) and then it contributed to the vascular supply of the medial ICRVs. The offshoots from the caudal tibial artery penetrated the epiphyseal hyaline cartilage, became EVCs and were a source of PEVs to the caudal periphery of

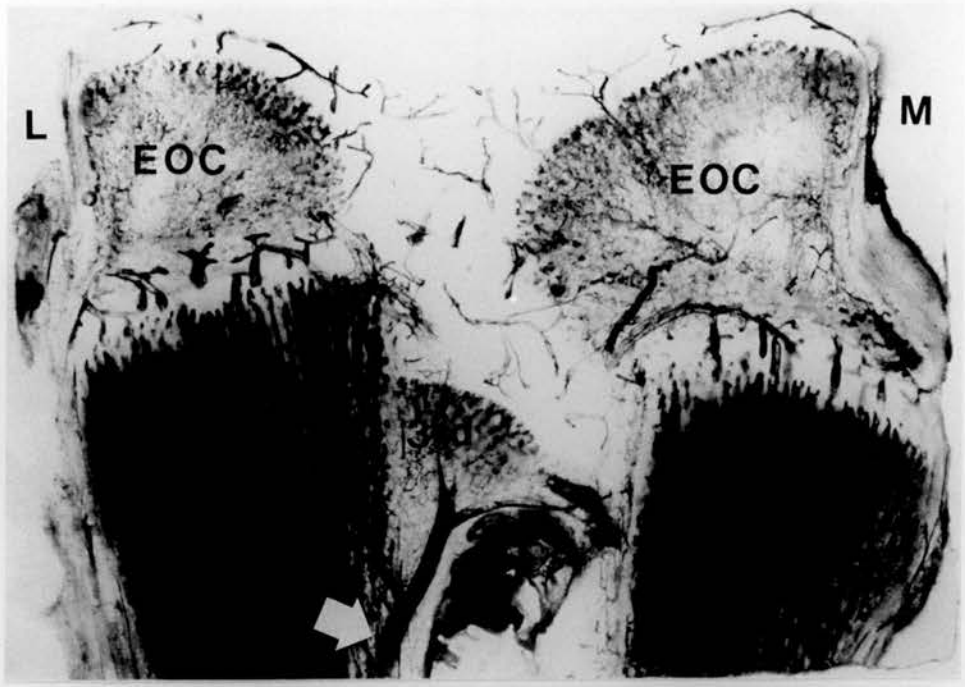


Fig 52.The distal tibiotarsus from a 3 week old S line. The medial EOC is larger than the lateral. A branch of the cranial tibial artery (arrowed) enters the cartilaginous epiphysis and then subdivides to supply the EOCs and the principal EVCs of the vascular crescent. 1mm slab x16.

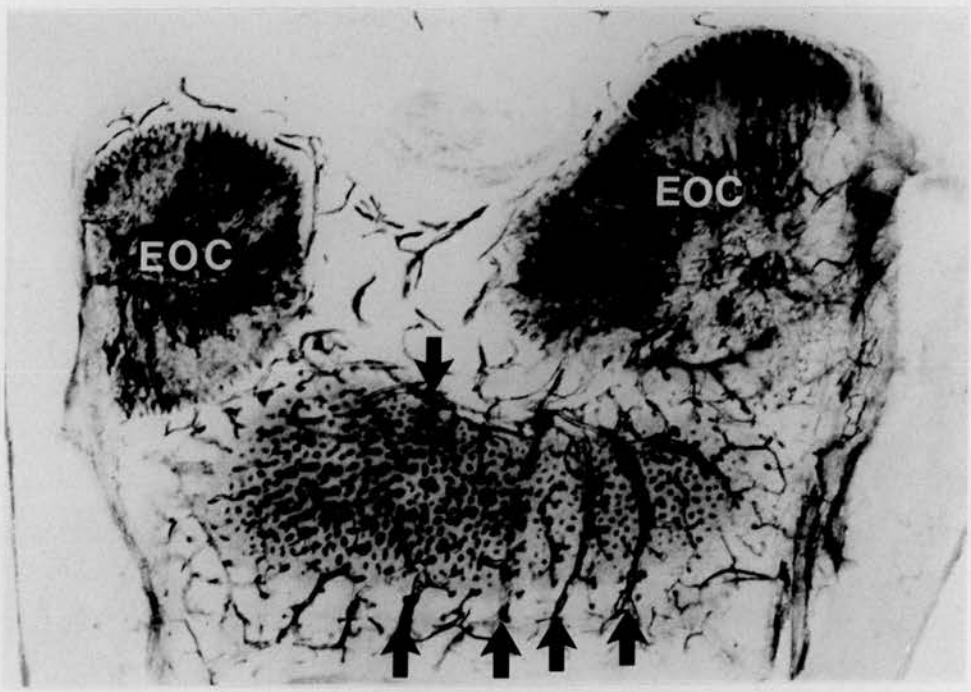


Fig 53. The distal tibiotarsus from a 3 week old S line. The principal EVCs (arrowed) are derived from the subdivisions of the branch of the cranial tibial artery. They run axially in a caudal direction through the cartilaginous epiphysis and parallel with the physis. 1mm slab x20.

the physeal cartilage.

In the condyles it was typical to observe branches from the principal EVCs supplying the underlying physis with PEVs, but the overlying thick cartilage of the condyles was maintained by EVCs from the medial and lateral ICRVs. The medial and lateral periphery of the distal tibiotarsal physis and cartilaginous epiphysis was supplied by EVCs and PEVs derived from the medial and lateral ICRVs.

The young bird

In day old specimens all three EOCs were present. The metaphysis of the distal tibiotarsus contained a cartilage cone encircled by invading MVs, which had already formed arrays around its periphery. The cartilage cone was penetrated by elongated transphyseal PEVs.

MV arrays rapidly spread across the metaphysis, so that by two days of age the only lack of continuity in the arrays was in the mid-caudal metaphysis. Transphyseal PEVs were now only present in the caudal metaphysis.

There was delayed MV array formation in four metaphyses between two and seven days of age. In each of these distal tibiotarsi a cartilage cone was still present in one of the condyles. Transphyseal PEVs were no longer present in the retained physeal cartilage core. The retained cores of metaphyseal cartilage occurred in three medial condyles (Fig 57) and one lateral condyle. By nine days of age all specimens had

Fig 54. The distal tibiotarsus from a 3 week old S line. The principal EVCs (arrowed) supply PEVs to the underlying physis and EVCs to the overlying epiphysis. 1mm slab x16.

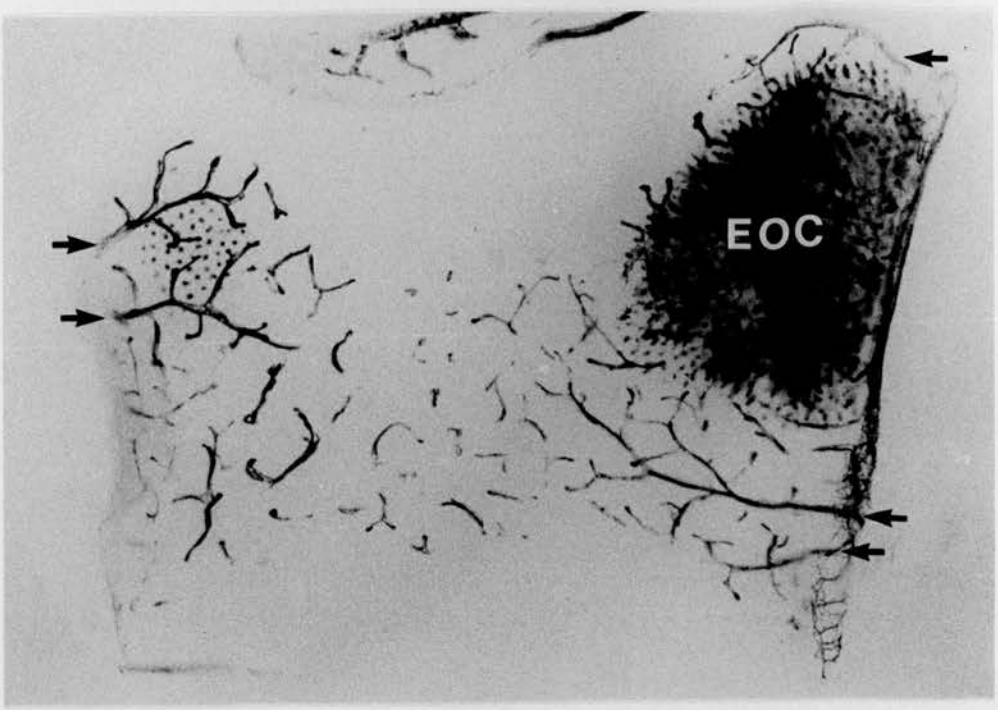


Fig 55. The distal tibiotarsus from a 3 week old S line. The cartilaginous epiphysis of the condyles is supplied by EVCs (arrowed) from the ECRVs. 1mm slab x16.

Fig 56. The distal tibiotarsus from a 6 week old S line. A branch of the caudal tibial artery (arrow) crosses the caudal surface of the distal tibiotarsus and gives rise to numerous offshoots which form EVCs in the periphery of the cartilaginous epiphysis. 1mm slab x10.

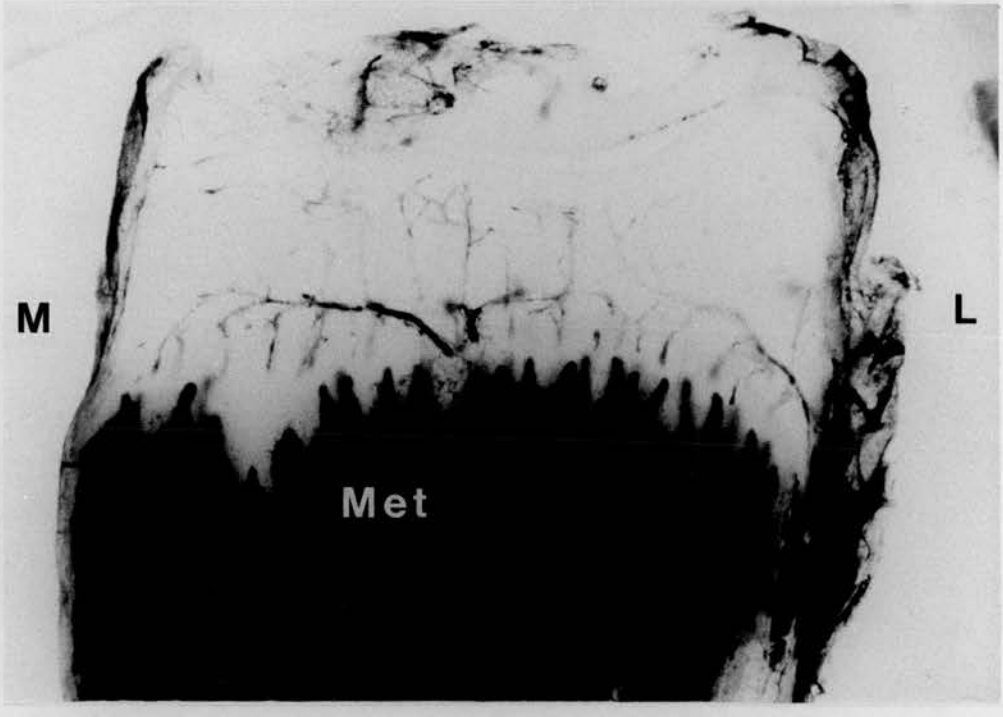


Fig 57. The distal tibiotarsus from a 7 day old S line. There is disturbed MV invasion of the metaphysis in the medial condyle. 1mm slab x 25.

fully functional arrays of MVs across metaphyses.

Epiphyseal ossification centres

In birds of seven days of age the medial EOC was growing rapidly and extending laterally. The periphery of the medial EOC, which consisted of tufts of vessels eroding the cartilage, was uneven. The margin of the slower growing lateral centre was uniform and regular.

The distal surface of the third EOC was level with the MV arrays of the metaphysis. The absence of a physeal cartilage and PEVs around the third EOC enabled it to be easily distinguished from the metaphysis of the distal tibiotarsus.

The paired condylar EOCs in young birds were surrounded by a "halo" of EVCs. As the ossification centres expanded in size these EVCs were overrun. The "halo" still persisted around the slower growing lateral EOC at twenty eight days of age.

The condylar EOCs by day 42 were much larger, conforming to the geometric shape of the surrounding cartilaginous epiphysis. The larger medial EOC was only separated from the articular surface by a narrow layer of hyaline cartilage (Fig 58).

In some 42 day old specimans there was virtual contact between the expanding lateral EOC and third EOC (Fig 59). By seventy days of age all three EOCs were united to form an ossified epiphysis. There was a narrow layer of epiphyseal hyaline cartilage adjacent to the physis and some remnants around the periphery of the ossified epiphysis. The narrow layer of hyaline

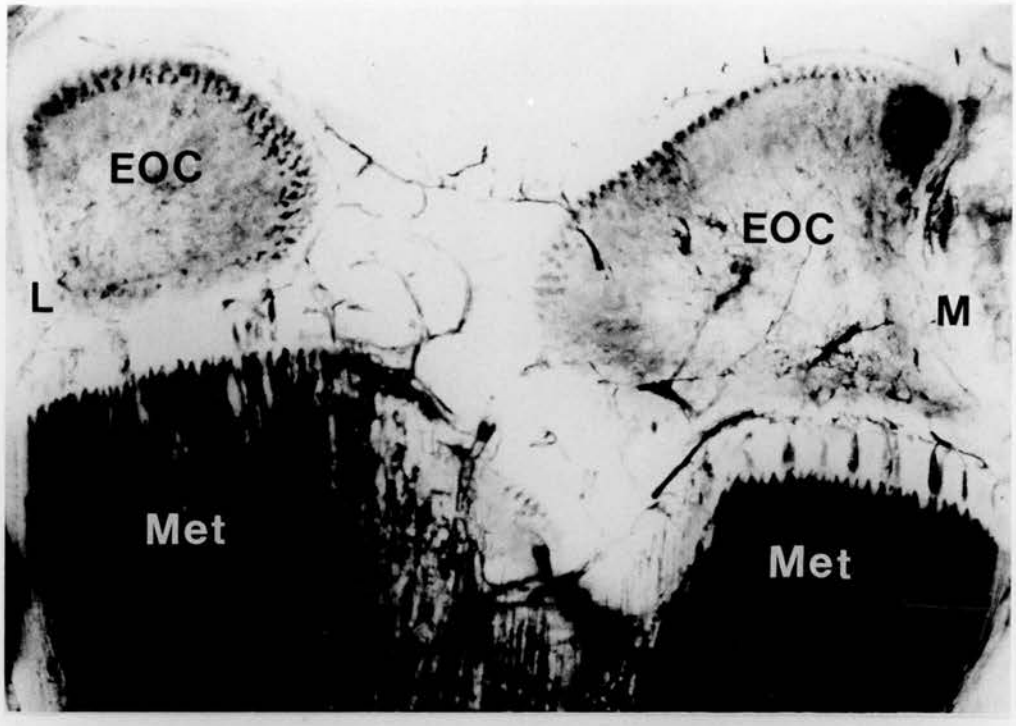


Fig 58. The distal tibiotarsus from a 4 week old S line. The medial EOC is larger than the lateral EOC. 1mm slab x16.

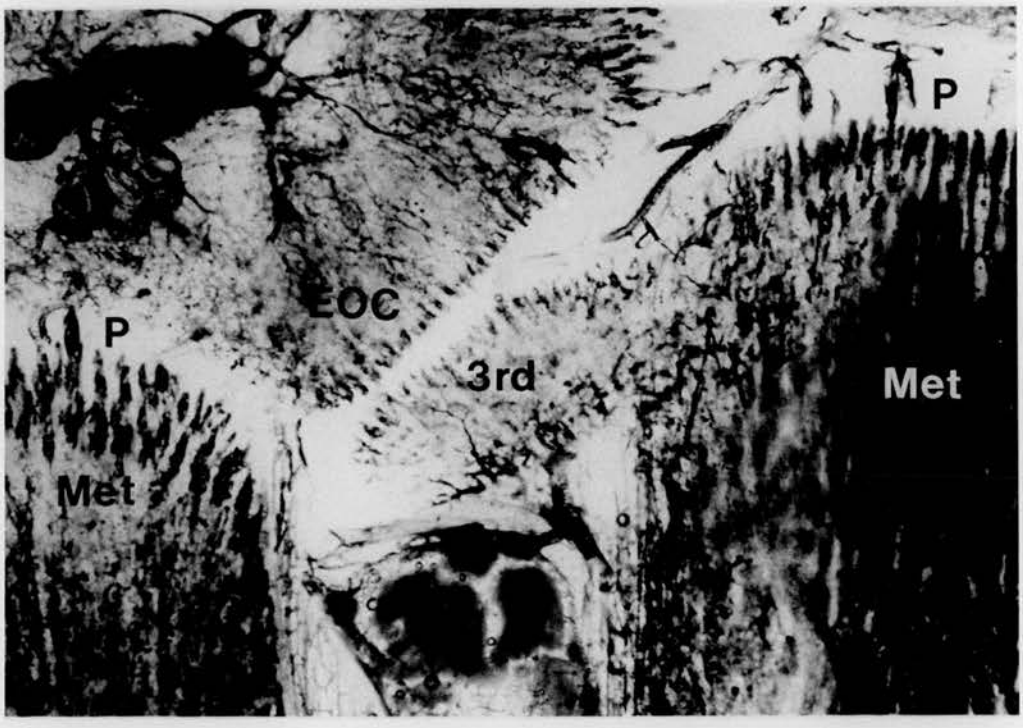


Fig 59.The distal tibiotarsus from a 6 week old S line. The expanding medial and third EOCs are about to make contact. 1mm slab x25.

cartilage conducted EVCs which supplied PEVs to the physeal cartilage (Fig 61). The EVCs were from the same sources as in the younger birds but anastomoses occurred between them and the vessels in the ossified epiphysis.

Physeal Closure

In birds of 70 days of age the PEVs were shorter and more widely spaced. By 105 days of age all the EVCs were incorporated into the ossified epiphysis. The males had no PEVs and only a narrow layer of physeal cartilage separated the metaphysis from the ossified epiphysis. The entire cartilaginous epiphysis was ossified (Fig 60). The female birds of 105 days of age already demonstrated the start of physeal closure with some MVs having crossed the physis into the ossified epiphysis. There was no physeal cartilage in the distal tibiotarsus of either male or female birds by 140 days of age.

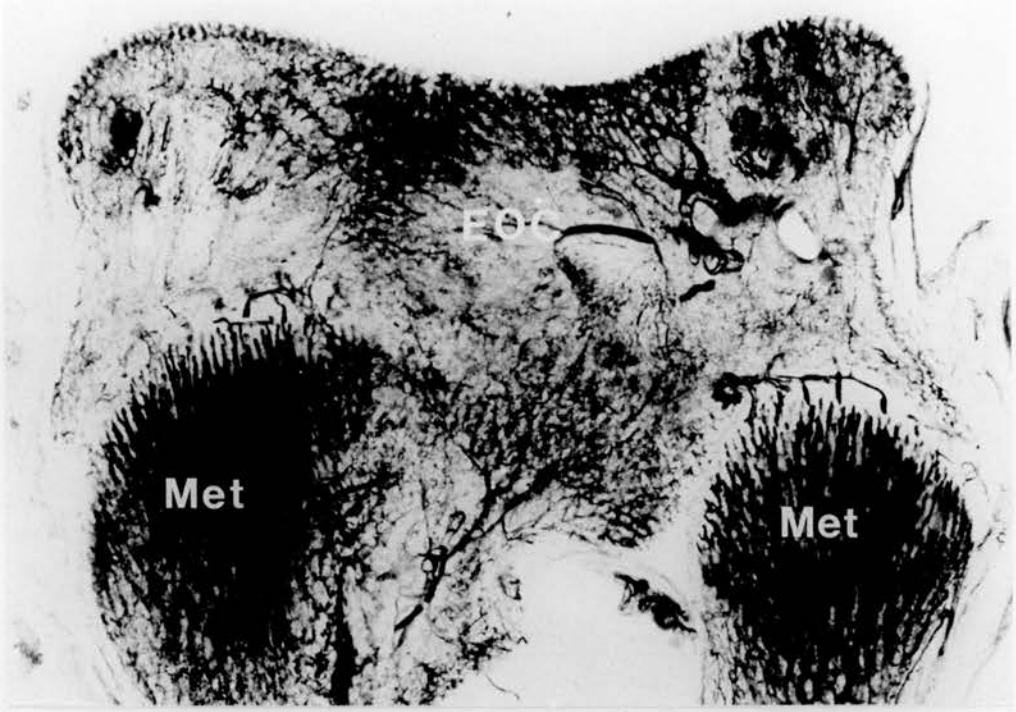


Fig 60. In the caudal view of this distal tibiotarsus from a 15 week old S line the three EOCs have united to form an ossified epiphysis. 1mm slab x10.

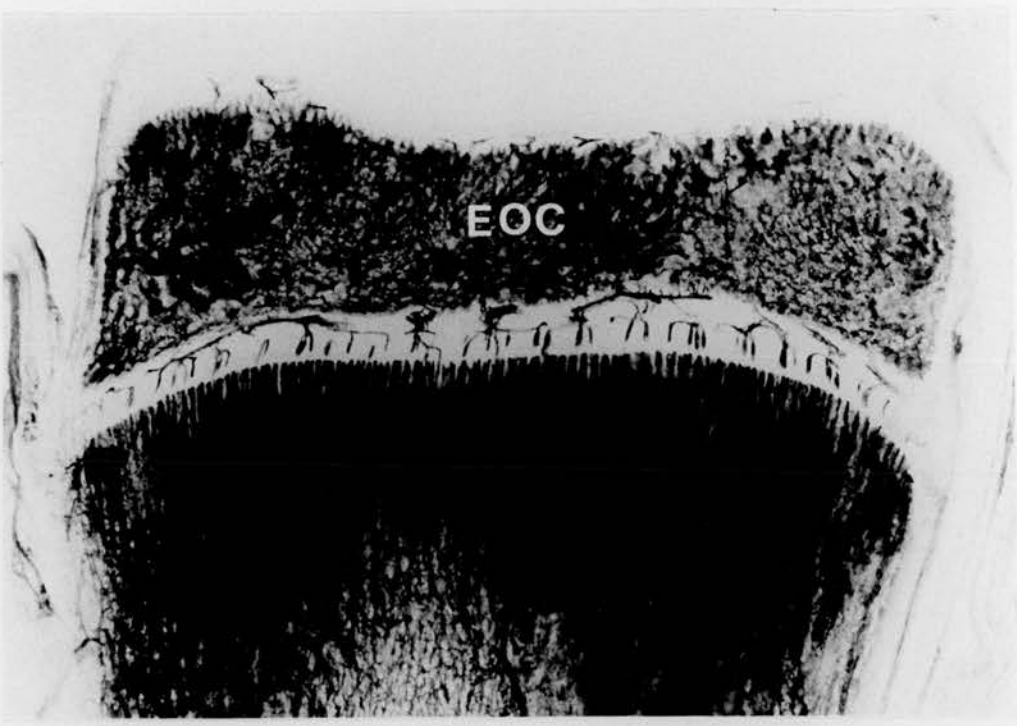


Fig 61. The distal tibiotarsus from a 10 week old S line. EVCs in the remaining cartilaginous epiphysis are anastomosing with vessels in the overlying ossified epiphysis. 1mm slab x10.

A pattern of EVCs was observed in all the bone extremities examined. The pattern of EVCs in younger birds was a simplified form of that seen in older individuals. Levene (1964) in mammalian studies also found that the pattern of EVCs in the newborn was a simplified, less extensive form of that occurring in older animals. In the present study the thicker the epiphyseal hyaline cartilage the more frequently were blind ending EVCs observed. This is possibly due to a reduction in the efficiency of intermittent compression aiding diffusion in thicker cartilage with a greater vascular input being required.

There were no anastomoses between the EVCs in the cartilaginous epiphyses, apart from in the proximal tarsometatarsus. The presence of vascular connections between the EVCs of the tarsometatarsus is probably due to the way in which this extremity evolved, as is discussed below.

The PEVs in all the physes were spaced in a regular and uniform manner. In each physis the PEVs tended to be equal in size and depth of penetration. This should have resulted in the maintenance of a uniform concentration of both nutrients and metabolites around the chondrocytes in the physis. The growth apparatus of the physis is extremely sensitive to oxygen tension (Brighton et al, 1969). These conditions should be ideal for consistent and controlled growth by the physeal cartilage.

The MVs were all of similar size and were arranged in well ordered, even arrays. They tended to penetrate the physis to a

constant depth. The MVs are required to be present before endochondral ossification can be initiated (Hladikova, 1981). The regular, even MV arrays would tend to bring about a steady rate of cartilage resorbtion and bone deposition.

In the vertebral bodies of the chick, parallel capillary tunnels of penetrating MVs do not occur, and there is only an undulating contour of vessels (Crissman and Low, 1974). The growth rate of the vertebral bodies is slow compared to the long bones of the pelvic limbs. In the bone extremities of the present study the structural arrangement of the MVs would increase the total surface area of vessels in contact with the calcified matrix, allowing a more rapid rate of osteogenesis.

The region supplied by an EVC system or group of EVCs from the same source was consistent between birds of the same age. The regional specificity of the EVCs was maintained with modifications throughout the development of the skeleton. A similar picture occurs in other species and Trueta (1957) also reported changes in the vascular pattern of the femoral head in man during growth. In the present study it became apparent that if the supply of EVCs from one source was inadequate then EVCs from an adjacent system could extend into what otherwise would be a poorly perfused area. This suggests that EVCs are able to respond to local stimuli and do not follow a totally programmed pattern of development.

There were transphyseal PEVs in all the extremities of day old birds. The PEVs extended deeply into the cone of metaphyseal cartilage, but were only present until a complete array of MVs had formed across the metaphysis. The presence of MVs appeared to

block the penetration of PEVs beyond the hypertrophic zone of chondrocytes. The more extensive the metaphysis the longer it took for an entire array of MVs to form. After a complete array had formed, only infrequently did PEVs cross the physis to then make vascular contact with MVs. The normal process of chondrocyte hypertrophy and calcification of the matrix appeared to "close" the metaphyseal end of PEVs. The completed array of MVs in young birds overlay a cone of avascular cartilage. The persistence of this cartilage was probably a result of the resistance of uncalcified cartilage to resorption (Silvestini et al, 1979). Its eventual removal is probably mediated by macrophage type cells.

Towards the end of the growth period there was a reduction in the number and size of PEVs. This coincided with a decrease in the apparent extent of the EVCs and there were fewer blind ending branches from established EVCs. The cartilaginous epiphysis would now have ceased growing. When the MVs advanced across the physis to ossify the cartilaginous epiphysis patent EVCs became less numerous. The EVCs underwent a process of closure due to vascular occlusion. Their function of maintaining the epiphyseal hyaline cartilage would not be required in an ossified epiphysis with its more diverse vascular supplies.

It was apparent that if the process of EVC occlusion and epiphyseal ossification became out of step then large areas of avascular cartilage would result. Such avascular areas of cartilage would only be slowly eroded by MVs (see experiment 10).

Chondrocytes in avascular cartilage would die and the matrix would undergo degeneration. Lesions occurring in some femoral

trochanters were of this type, similar to a form of osteochondrosis reported in the trochanter of broiler type fowls (Duff, 1985a).

In the present study there were areas of thickened physeal cartilage in the physis of the proximal femur and tibiotarsus. There was a delay in MV invasion of the physeal cartilage and a retention of prehypertrophied chondrocytes, typical of dyschondroplasia (Poulos et al, 1978; Duff, 1984a). These small lesions in the S line fowls did not cause clinical signs. Minor areas of dyschondroplasia, which did not cause clinical signs have been reported in wild type pigs (Fell et al, 1967). The occurrence of such small aberrations in the normal process of endochondral ossification is probably not infrequent. In relatively slow growing S line fowls lesions such as these probably repair rapidly before more severe pathology develops.

In young birds there was no calcification of the cartilage matrix which surrounded the transphyseal PEVs. Physeal cartilage underwent calcification only when MVs were present. This suggests that during normal endochondral ossification MVs are required before the hypertrophied matrix can calcify.

Anastomoses between transphyseal PEVs and MVs only occurred in birds under fourteen days of age. Howlett (1980) noted the absence of PEV/MV anastomoses in a study of seven week old fowl.

The presence of the EOC in half of the proximal tibiotarsi by six weeks of age was earlier than reported by Hogg (1980). This author however studied a different breed, the golden comet, and examined a smaller group of birds. In the present study the EOC

developed in an area which was well perfused with EVCs, many of which were blind ending and enlarged. Agrawal et al (1984) considered that hypertrophy is induced in the chondrocytes at the end of the EVCs of the forming EOC. The development of the EOCs in man occurs at the site of the cartilage canal glomeruli. Wilsman and van Sickle (1970) made similar observations in the pup stating that; "At the site of the presumptive epiphyseal ossification centre dense foci were observed, which were in the vicinity of the glomerular type termination to some of the cartilage canals".

EOC formation in the avian proximal tibiotarsus resembled EOC formation in the rat and rabbit as reported by Kugler et al (1979). Occasionally in the proximal tibiotarsus, anastomoses occurred between EVCs supplying the EOC and those of the cartilage epiphysis. Indeed a similar pattern was noted in the proximal femur of the human foetus where EVC anastomoses only occurred when the EOC had formed (Crock, 1967)

In the present study the even contour of expanding EOCs was sometimes interrupted by local invaginations of epiphyseal hyaline cartilage. This fold of cartilage carried an EVC which supplied the EOC. This EVC appeared to function primarily as a source to the EOC and did not branch to supply the epiphyseal hyaline cartilage. Similar pits and grooves of epiphyseal hyaline cartilage, which transmit EVCs into the developing EOC. have been reported in the growing pig (Visco and Kincaid, 1983).

The fully ossified epiphysis in the growing fowl was very similar to that in the growing mammal. The most apparent

difference between species was the persistence of a thin layer of epiphyseal hyaline cartilage between the ossified epiphysis and the physeal cartilage in the fowl. This stratum of cartilage carried EVCs which formed the PEVs and anastomosed with vessels from the ossified epiphysis.

The end of growth, identified by MVs crossing the physeal cartilage, occurred at a younger age in female birds. Latimer (1927) found that the femur and tibiotarsus of female fowls ceased to grow at 116 and 112 days respectively. The corresponding ages for physeal closure in males was 144 and 140 days. Earlier skeletal maturity in female birds was also noted by Itakura et al (1975). In the present study physeal closure was also earlier in the females than males, with closure commencing in the latter at 20 weeks of age.

The term junctional canal has been used to refer to cartilage canals situated between the cartilaginous epiphysis and physis. The function of the junctional canals is to supply the PEVs (Duff, 1984a; Lacey and Huffer, 1984). In this study EVCs were apparent which specifically formed PEVs. These EVCs did not run adjacent to the physeal cartilage for any great distance and did not differ in appearence from the other EVCs. There was no readily identifiable junctional canals.

The cells which multiply to increase the size of the cartilaginous epiphysis in the proximal tibiotarsus of the fowl are found in the perichondrium and cartilage canals (Lutfi, 1970b). The systems of arborizing EVCs were all relatively evenly spaced throughout the cartilaginous epiphysis enabling it to

increase in size uniformly and so maintain joint congruity.

The proximal femur is a common site of orthopaedic disease in many species. Many of the conditions are considered to be related to blood supply. The blood supply to the developing femoral head in man has been described in detail (Trueta, 1957). Sevitt and Thompson (1965) also demonstrated a similar regional specificity of vessels in the femoral head of man. In the newly born infant the proximal femur shares one common cartilaginous epiphysis (Ogden, 1981) similar to that of the developing fowl. Vessels in the capital femoral ligament vary in their contribution to the overall vascularity of the femoral head, and are associated with the development and repair of lesions in Perthes disease (Mickelson et al, 1980). These same vessels in the fowl make a significant contribution to the blood supply of the cartilaginous epiphysis of the femoral head throughout growth. The EVCs from the capital femoral ligament have even been reported as forming an epiphyseal ossification centre in an attempt to repair dyschondroplasia in the avian femoral head (Duff, 1984c; Duff and Hocking, 1986).

Some features of the vascular pattern of the femoral head in man persist throughout life (Trueta and Harrison, 1953). Similarly in S line fowls, some of the vessels which had supplied the cartilaginous epiphysis during growth remained in skeletally mature individuals.

There are a lot of similarities between the vascular supply

to the femoral head in the fowl and that of other species. Fitzgerald (1961) considered that in the dog the plexus of vessels in the synovial membrane were one of the principal vascular supplies to the femoral heads. These vessels are the same as the ICRVs in the present study of the fowl. The importance of the ICRVs in the pup was emphasised by Bassett et al (1967). He described the retinacular vessels as directly penetrating the epiphyseal hyaline cartilage via the articular cartilage. The cat also has a similar vascular pattern in the proximal femur, with vessels from the capital femoral ligament and intracapsular ring (Pohlymeyer, 1981). Harty (1953) described Babcocks triangle as supplying vessels to and from the head of femur in man. This structure corresponds in situation and function to the perichondrial ring of the proximal femur in the fowl.

The different origins of the EVCs affects their ability to withstand insult. The uptake of radiactive phosphorus (32P) in different areas of the femoral head was reduced by elevating the intra-aticular pressure and compromising the ICRVs (Bassett et al, 1967). In other species with similar vascular anatomy ICRVs would also be vulnerable to elevated intra-articular pressure. In the fowl the medial femoral head was identified by Duff (1984a and 1984b) as an area susceptible to EVC occlusion and the present study shows that the medial femoral head is supplied by ICRVs. This suggests a possible pathogenesis of the lesions in the medial femoral head of the fowl. Synovitis may lead to elevated intra-articular pressure causing compression of the ICRVs. The resulting vascular stasis may cause the formation of thrombi

culminating in EVC occlusion.

In the normal femoral head in man the extent of the epiphysis which is supplied by vessels from the capital femoral ligament, varies (Werthiemer and Lopez, 1971). This suggests that compensation for variation in the area supplied by an EVC system is not unique to the fowl.

The vessels in the capsule of the hip joint were identified by Wolcott (1943) as being important in the development of the proximal femur. In the dog the subsynovial vascular plexus of intracapsular vessels is well developed on the cranial and caudal surfaces of the femoral neck (Kaderly et al, 1983). The corresponding regions of the proximal femur in the fowl were supplied, cranially by a large retinacular vessel and caudally from a large vessel to the perichondrial ring. The retinacular tissue and perichondrial ring at these sites was well vascularised and made a significant contribution to the epiphyseal blood supply.

The proximal epiphysis of the tibia in the sheep, goat, cat and rabbit all contain vascular canals for a considerable period of time before the epiphyseal ossification centre starts to grow (Levene. 1964). A similar situation exists in the fowl. The predominant source of EVCs to the epiphyseal hyaline cartilage in the proximal tibia of the sheep penetrate the cartilage at a small circumscribed area immediately above the tibial tubercle and deep to the patellar tendon (Levene. 1964). This description is remarkably similar to that of the intra-articular vessel that penetrates the avian tibiotarsus at the intercondylar eminence. The peripheral canals described by Levene (1964) resemble the EVCs supplied by ICRVs and ECRVs in the proximal tibiotarsus of the fowl.

The epiphysis of the proximal tibia in the rabbit is similar to the fowl in being supplied by two anterior intercondylar arteries (Brookes and Harrison. 1957). In man the blood supply to the developing proximal tibia also shares similarities with the fowl. There are intercondylar arteries which penetrate the tibia via the intercondylar eminence in man (Scapinelli, 1968). The vessels in the cartilaginous epiphysis of man are independant and have well defined areas of distribution. The development of the epiphyseal ossification centre results in anastomoses of the epiphyseal vasculature (Scapinelli, 1968). Anastomoses of EVCs was only seen to occur in proximal tibiotarsi of fowls after the

formation of the EOC.

Hogg (1980) considers that the only true secondary ossification centre to occur in the fowl is in the epiphysis of the proximal tibia. The cranial position of the EOC in the cnemial crest at the site of attachment of the patellar tendon led Parsons (1904 and 1905) to suggest that it is a traction epiphysis in mammals. Indeed Hogg (1977) stated that the proximal tibial EOC may represent a further strengthening device for the insertion of the quadriceps femoris muscle. Parson's theory (1904 and 1905) suggested that fused sesamoids gave rise to ossified traction epiphysi. This is supported by Burnett and Lewis (1958), who examined the sesamoids present in the joints of long bones in a variety of species. They concluded that it is reasonable to assume that traction epiphyses correspond in form and position to sesamoids and that one type of structure has probably given rise to the other.

In man the tibial tuberosity develops as an outgrowth of the proximal chondroepiphysis in association with an ingrowth of fibro-vascular tissue. During the foetal period this outgrowth "migrates" anterodistally to the tibial metaphysis. The fibrovascular ingrowth remains between and separates the proximal tibial growth plate from the tibial tuberosity. About four to six months after birth, a growth plate develops under the tibial tuberosity and with time acquires structural adaptions to accommodate large tensile stresses (Ogden et al, 1974). The tibial tuberosity is separated from the diaphysis by a fibrous growth plate, which is a structural response to its environment.

The fibrous plate functions in the same manner as a cartilaginous physis.

In tissue culture primitive fibroblasts respond to environment and physical forces to differentiate into three distinct tissue types. These are bone, cartilage and fibrous tissue (Bassett 1962). The development of an EOC in a traction epiphysis may be the product of osteogenesis initiated by the action of physical forces and environmental changes on the fibroblasts of the EVCs. This study confirmed that the EOC in the proximal tibiotarsus of the fowl was restricted to the cnemial crest and did not incorporate the epiphyseal hyaline cartilage of the condyles. It appears reasonable to liken the EOC in the anterior epiphysis of the tibiotarsus to the EOC in the tibial tuberosity of other species.

In the chick embryo the tarsal bones fuse with the tibia and metatarsus to form the tibiotarsus and tarsometatarsus (Gegenbaur, 1864. cited by Neilson, 1963). The tarsal elements of the proximal tarsometatarsus insert as a wedge across the three metatarsi. Vascular ingrowth between the tarsal elements and the metatarsi appeared to follow the line between the epiphysis and physis (Neilson, 1963).

In the present study the lattice like arrangement of EVCs in the proximal tarsometatarsus lay in a plane parallel with the physis. All the anastomoses between EVCs occurred in this plane. This vascular stratum may be the demarcation between the tarsal elements and the metatarsi. If this is the case it is conceivable that the EVCs have evolved from retinacular vessels, which were present when the tarsus and metatarsi were separate entities. This would explain the frequent vascular connections between EVCs which in all other cartilaginous epiphyses were end arterial systems. The blood supply to much of the cartilaginous epiphysis is unusual in that it arises from the two foraminal vessels situated in the metaphysis. This feature again is a relic of evolution caused by the synostoses of the three metatarsi trapping the vessels between their respective metaphyses.

The proximal tarsometatarsus contained an epiphyseal ossification centre in the middle of the cartilaginous epiphysis, which was present from hatching. This EOC is considered to be the

ossification centre of a tarsal element (Romanoff, 1960; Hogg, 1980), and was described by Morse (1872) as a distal tarsal ossicle which ankylosed with the metatarsi. Bruce et al (1946) in a radiographic study of the proximal tarsometatarsus described the EOC as lens shaped in the eight week old bird. In the present study, the EOC was obserbed to enlarge into the medial cotyle prior to its lateral extension.

The articular surface of the tarsometatarsus supports the entire weight of the bird when it is standing on one leg (Barnett, 1954). It appeared in the present study, that if extremes of normal intertarsal angulation were present then there would be greatly increased loading on the medial or lateral cotyle and its respective metaphysis. Compressive pressure was maintained with springs across the distal femur and proximal tibia in the growing rabbit (Trueta and Trias, 1961). In the early stages of this experiment only the metaphysis were affected. There was a lack of progression of MVs into the physeal cartilage. In the newly hatched chicks, in the present study, there was a marked variation in intertarsal angulation with both varus and valgus being recorded. The tibiotarsus of the chick embryo can become bent as a result of intrinsic and extrinsic mechanical stress induced experimentally by limb bud reorientation (Amprino, 1985). It is conceviable that similar stress patterns may be induced in the limbs by the position of the embryo in the shell; resulting in the variety of intertarsal angulations found in the chicks at hatching. This would result in an increase in load bearing by either the medial or lateral metaphysis and accounting for delayed

MV invasion of the cartilaginous model in these areas.

There are many similarities in the blood supply to the developing avian femur and that occurring in other species. The distal femur of the growing pig was examined by Hill et al (1985), who concentrated on the early, mainly cartilaginous epiphysis. The periphery of the epiphysis was supplied by medial and lateral perichondrial vessels, similar to those in the distal femur of the fowl. In one of Hill et al's (1985) radiographs there was an EVC from the perichondrial ring supplying PEVs to the physeal cartilage in the pig. This appeared very similar to the EVCs from the lateral perichondrial ring in the fowl. In another of their radiographs the intercondylar vessels which supplied EVCs to the underlying epiphysis were readily apparent. This area is the femoral attachment of the cruciate ligaments in the pig and the fowl. In the fowl, intercondylar vessels were present but they only functioned to supply the cruciate ligaments and did not form EVCs.

In man the middle genicular artery, which arises from the popliteal artery, penetrates the knee joint in the posterior intercondylar notch, and is responsible for much of the blood supply to the epiphysis of the distal femur (Scapinelli, 1968). The deep fovea in the posterior intercondylar region of the distal femur is where the arterial branches penetrate this bone extremity (Rogers and Gladstone, 1950).

In the young rat the intercondylar notch in the caudal

epiphysis is the site of entry of a vessel which, divides to extend branches cranially supplying the cartilage of the medial and lateral condyles (Brashear, 1963).

In the distal femur of the rabbit a vessel, which is a branch of the popliteal artery, penetrates the caudal aspect of the distal femur (Brookes and Harrison, 1957). These vessels in the rat, rabbit and man correspond to the intercondylar artery on the caudal aspect of the distal femur in the fowl. In the present study the intercondylar artery penetrates the posterior aspect of the distal femur to supply the "horseshoe" of principal EVCs.

The vessels on the medial and lateral surfaces of the distal femur in man appear to be similar in structure and function to the retinacular vessels in the fowl.

The cartilaginous epiphysis of the distal tibiotarsus in the fowl is considered to contain two ossification centres that unite. The EOCs in the distal tibiotarsus were present in the day old chicks. In the 18 day old embryo there is histological evidence of bone formation in the cartilaginous epiphysis of the distal tibiotarsus (Navagiri and Dubey, 1975). The ossification centres represent a proximal row of tarsal elements that have become incorporated into the epiphysis of the distal tibia. Hogg (1980) confirmed that two centres exist in the cartilaginous epiphysis of the distal tibiotarsus and he criticized Franceschini (1967) who reported three. Other reports have stated that there are only two ossification centres present (Bruce et al, 1946; Church and Johnson, 1964 and Navagiri and Dubey, 1975). In studies of embryonic chicks Romanoff (1960) described a third tarsal element, the intermedium. The intermedium became the ascending process of the astragulas on the cranial aspect of the distal tibiotarsus. Neilson (1963) in a diagrammatic representation of the talus delineated the "process ascendes", which corresponds to the astragulas as described by Romanoff (1960). The distal tibiotarsus in the quail also contains three fused tarsal elements. The three elements are fused by day twelve in the embryo and an ascending process extends proximally in a groove on the anterior face of the tibiotarsus (Lansdown, 1970). The later ossification of the ascending process had been noted by Morse

(1874).

Francheschini (1969) prepared serial sections from the distal tibiotarsus which were stained with alizarin. In the young bird he recognised the paired condylar EOCs and a third EOC in the "epiphyseal cartilaginous cone". This cartilage cone corresponds to the astragulas described earlier. Francheschini (1969) was unsure of the frequency of occurrence of the third EOC in the distal tibiotarsus. The present study has confirmed that the third EOC was a regular feature occurring in all specimens.

The third EOC of the distal tibiotarsus is situated at the distal point of attachment of the Pons supratendineus or Lig. transversum ossificatum. The ligament lies obliquely and restrains the tendon of the cranial tibial muscle. There will be considerable force transmitted from the tendon to this ligament. The force will cause a traction effect at the distal point of attachment of the ligament at the site of the third ossification centre. The fibrovascular layer of perichondrial tissue, which separates the third EOC from the metaphysis in the distal tibiotarsus of the fowl is a fibrous growth plate. Fibrous growth plates occur at the sites of traction epiphyses (Smith 1962a and 1962b). The third ossification centre although present, in what initially was a distinct tarsal element, may be a form of traction epiphysis similar to the tibial tuberosity.

Francheschini (1969) also noted that the medial EOC grew more rapidly than the lateral. This finding has been confirmed in the present study. The medial ICRVs form a more extensive system of EVCs through the medial condyle which supply the medial EOC. The

better vascularity of the medial condyle compared to the lateral is probably related to the more extensive medial EOC.

There was delayed MV invasion in the metaphysis of some of the young birds. This delay either occurred in the medial or lateral condyle and may have been a response to increased medial or lateral metaphyseal loading. The increased loading may originate from abnormal intertarsal angulation in the newly hatched chick.

EXPERIMENT 4:Broiler fowl fed ad libitum.

INTRODUCTION.

In experiment 3 the pattern of cartilage canals was established in the bone extremities of the developing S line fowl, which demonstrated a low incidence of developmental orthopaedic disease. The purpose of the present experiment was to record the vascular pattern in a different genotype of fowl, with a high incidence of developmental orthopaedic disease.

The broiler fowl (Fig 63), as a result of selection for fast growth varies markedly from the S line fowl (Fig 62). The skeleton of the broiler increases rapidly in size and the birds suffer a high incidence of dyschondroplasia (Bergman and Scheer, 1975), which can be defined as a focal failure of endochondral ossification. In the avian literature the term dyschondroplasia has been adopted for osteochondrosis of physeal cartilage, and is a better descriptive term (Olsson, 1978; Duff, 1984c). To differentiate the pathology of failed endochondral ossification of epiphyseal hyaline cartilage from that of physeal cartilage Duff (1985a) proposed that the term osteochondrosis be used for the former and dyschondroplasia for the latter. Osteochondrosis has been described as a spontaneous dyschondroplasia of unknown cause occurring in dogs, bulls, horses, turkeys, broilers, pigs and man (Craig and Riser, 1965; Poulos, 1978; Poulos et al, 1978; Reiland, 1978a and 1978b; Reiland et al, 1978b; Stromberg and

Rejno, 1978 and Murbarak and Carroll, 1982). Hill et al (1984) considered that the term osteochondrosis encompassed the resultant pathology of dyschondroplasia and osteochondrosis.

For the purposes of the present study osteochondrosis is considered to be a form of dyschondroplasia of the epiphyseal hyaline cartilage. The term osteochondrosis should be considered as a synonym for dyschondroplasia of the epiphyseal hyaline cartilage. Differences were reported by Levene (1964) in the pattern of cartilage canals in two different breeds of sheep. The present study aims to establish if there is a difference in the pattern of cartilage canals in two very different breeds (genotypes) of fowl, and if the pattern of canals is associated with orthopaedic disease in the growing skeleton.

MATERIAL AND METHODS.

The broiler chicks selected for this experiment were M4 strain birds, bred by D.B.Marshalls Ltd as broiler breeding stock (Fig 63). One hundred and twenty broiler chicks were reared from day old. They were fed a similar diet to the birds in experiment 3 and were kept under similar husbandry conditions. The birds were all weighed weekly. The number and age of these birds at each kill was the same as in the previous study, except for an additional kill of eight birds at 56 days. After killing, the birds were routinely weighed. Angulations of the intertarsal joint were recorded. After dissection the pelvic appendicular skeleton was radiographed in a Faxitron 804 using Kodak X-Omat RP film in a Kodak X-Omatic fine screen plate. Both AP and lateral views were taken. Estimates of torsion were recorded for the three long bones, by comparing the transverse axis of the proximal and distal articular surfaces (Duff and Thorp, 1985a and 1985b). Post mortem details, including age, sex weight, intertarsal angulation, long bone torsion and long bone length, for each bird is recorded in Appendix 2.

Bone extremities from four birds of each kill were processed in Polymaster resin, and the others were stored in 10% BNF. The Polymaster blocks containing the proximal and distal femurs, proximal and distal tibiotarsi and proximal tarsometatarsi were subsequently cut into slabs and examined. The identical format of this experiment and experiment three was to facilitate a direct comparison of the results.

Fig 62. Adult female S line fowl.

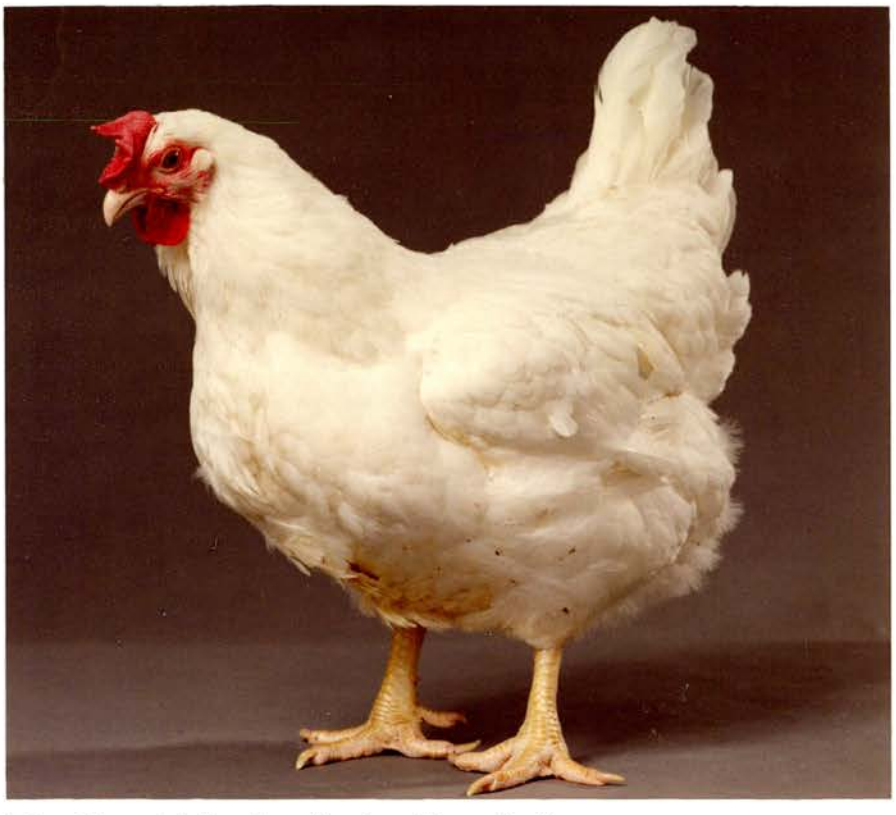


Fig 63. Adult female broiler fowl.

RESULTS.

Live weight gain.

The average live weight gain of the male and female birds in each group is presented in Fig 64. The number of birds remaining and contributing to the mean body weight decreased as the experiment progressed, because birds were killed through the experimental period. In both groups the male birds were of a heavier average weight than the females.

The pattern of weight gain in the ad libitum fed broilers produced an S shaped curve. This was due to an accelerating rate of weight gain in the four weeks post-hatching, then a period of constant rapid growth which was followed by decelerating growth from between ten and twelve weeks of age.

Bone length.

The femurs, tibiotarsi and tarsometatarsi of all the birds in experiments three and four had been radiographed. The length of each long bone was measured on the radiographs. The average length of each of the three long bones for each age group was calculated. In the broilers the tarsometatarsus was frequently slightly shorter than the femur. Tarsometatarsi, because of their similarity in length to the femurs, do not appear on the graphs of long bone length. There were rarely any differences in the length of the long bones in the right and left limbs from each bird. The average length of the long bones throughout the growth period is plotted in Fig 65a. At the end of the growth period the long

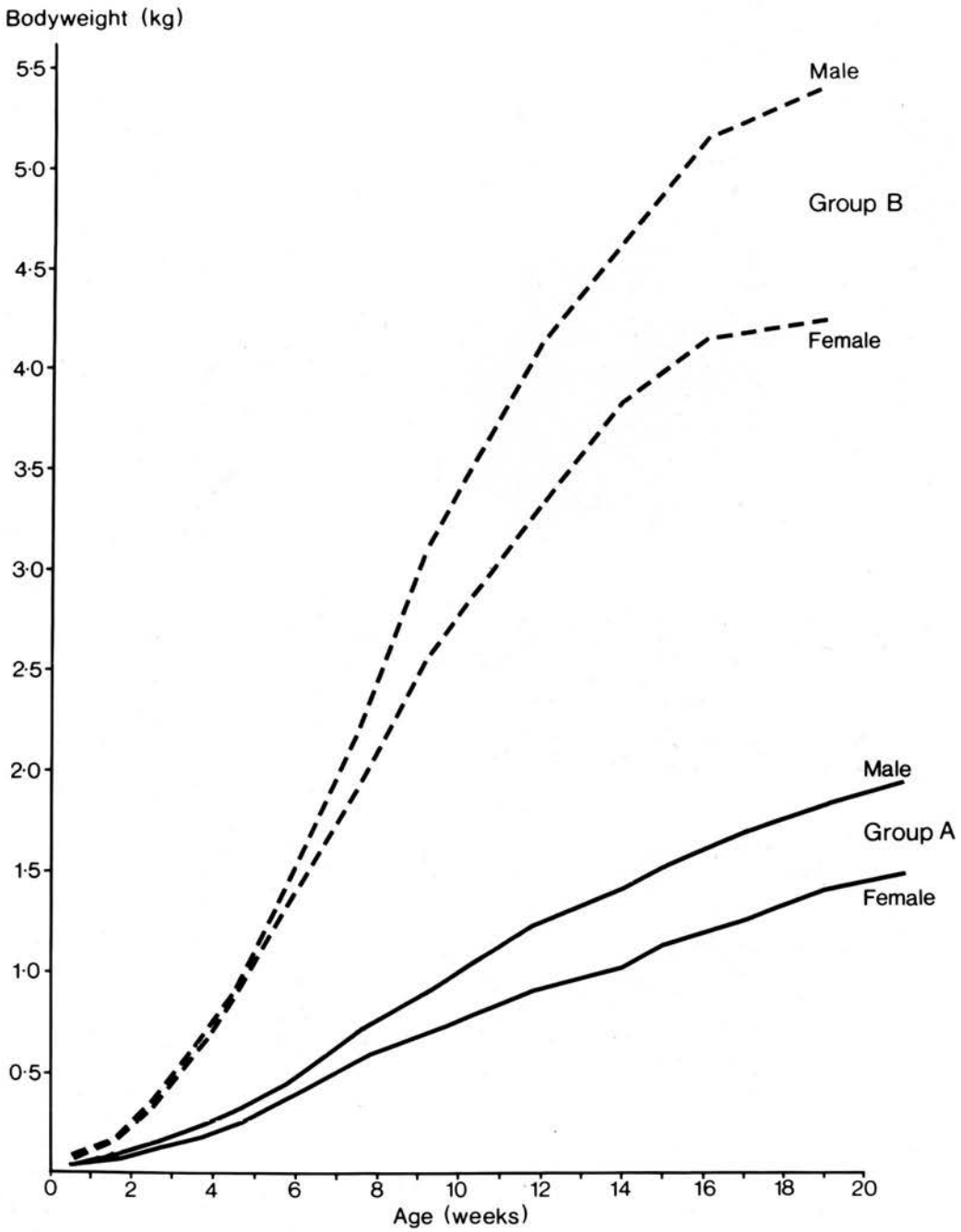


Fig 64. The average body weights of ad libitum fed S line (group A) and broiler (group B) fowl from day old to 20 weeks.

bones of the broilers were 10% longer.

Growth rate.

The difference in average bone length between successive kills was used to calculate the growth rate of the long bones. The growth rate was plotted against age in Fig 65b. The fastest growth rate (14.4mm per week) was recorded in the tibiotarsus of the broiler fowls between two and three weeks of age, during which time femurs of the broiler fowls grew 10.2 mm. The S line tibiotarsi, between two and three weeks of age, grew 10.4mm.

Limb angulation.

Estimates of intertarsal angulations of the broilers in experiment 4 are recorded in Appendix 2. In the broilers there was a wide range of intertarsal angulations with the majority of birds having a valgus deformity in the range of 5 to 15 degrees.

Bone torsion.

All torsional measurements are recorded in Appendix 2. The distal femurs were normally rotated externally relative to their proximal joint surface. In at least 97% of cases between 2 and 20 degrees external torsion was measured. Distal tibiotarsi were normally rotated externally, but internal torsion was present in a proportion of the broilers, especially in those birds of six to ten weeks of age. Distal tarsometatarsi were rotated internally relative to their proximal joint surface in all cases, apart from two broilers with external rotation of 0 and 6 degrees

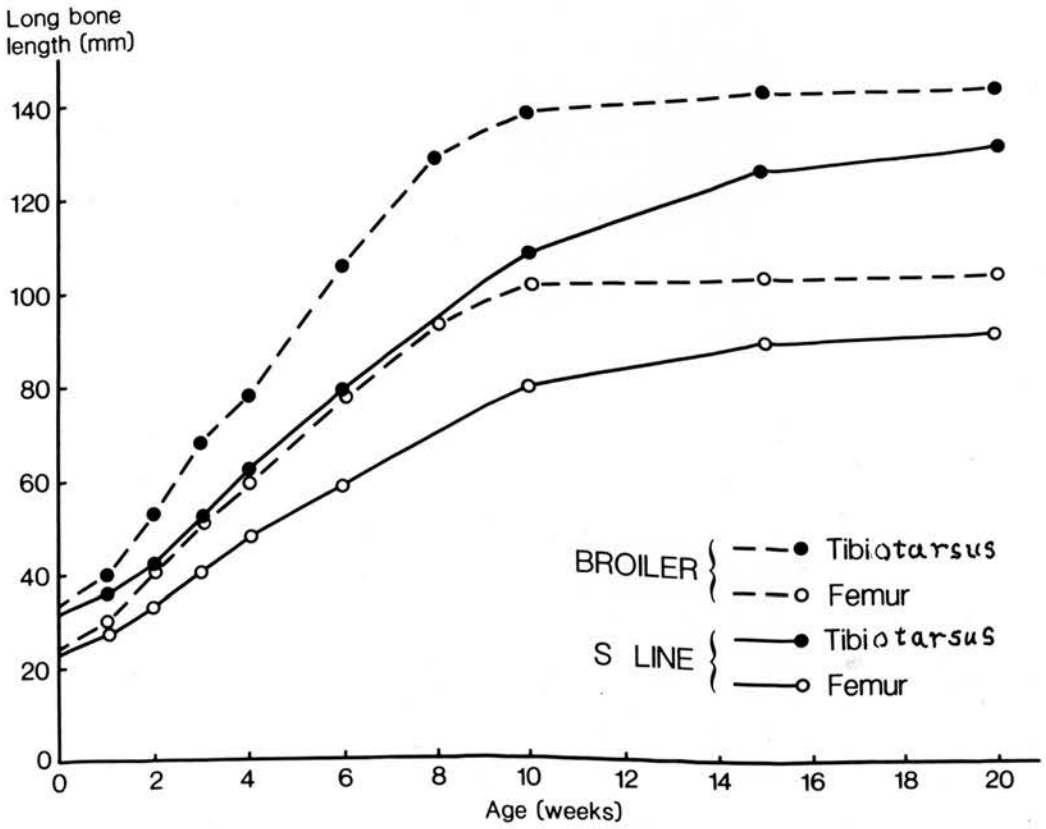


Fig 65a. The length of the tibiotarsus and femur in ad libitum fed broilers and S line fowls plotted against age.

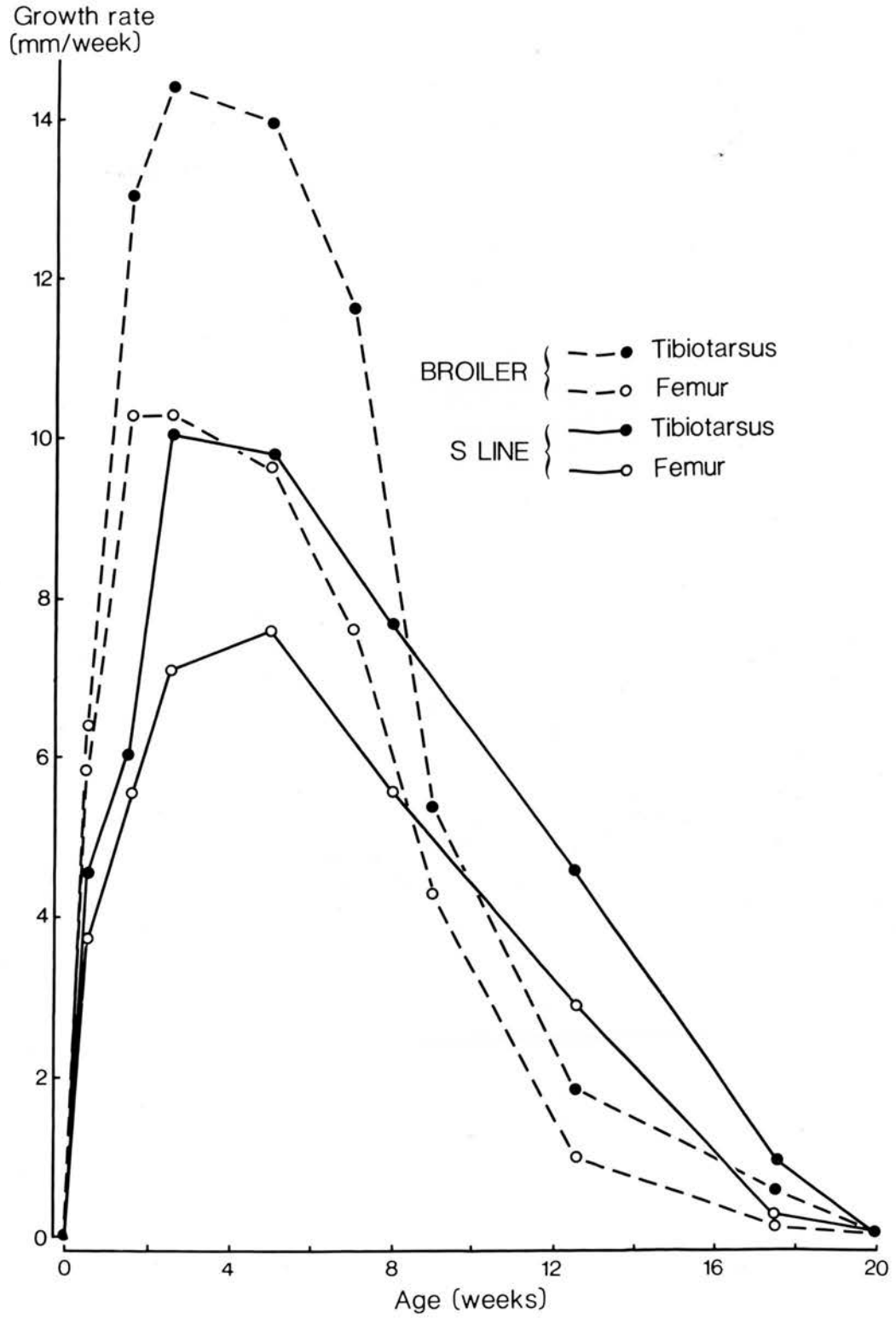


Fig 65b. The weekly growth rates of the femur and tibiotarsus in ad lib fed S line and broiler fowl.

respectively.

The vascular pattern of the bone extremities in ad libitum fed broilers.

The fundamental vascular pattern in all the cartilaginous epiphyses of the broilers was very similar to that found in the S line birds of the same age. In the broilers the EVCs were longer, branched more frequently and were more extensive. There were, from the broiler EVCs, a greater number of blind ending vascular buds in the epiphyseal hyaline cartilage. In birds of the same age the cartilaginous epiphyses were larger in the broilers.

In the broilers, at a number of sites, there were networks of EVCs in the epiphyseal hyaline cartilage directly under the articular cartilage. These sub-articular networks were most distinct in the caudal intercondylar region of the distal tibiotarsus (Fig 66). Where ICRVs were present on the articular surface there were no sub-articular networks of EVCs. Similar profusions of sub-articular EVCs, though not as manifest, occurred in the cartilaginous epiphyses of the proximal femoral neck, distal femoral condyles, proximal tibiotarsal condyles (Fig 68) and the cotyles of the proximal tarsometatarsus (Fig 67). Subarticular networks of EVCs were more extensive and obvious in the broiler fowls (compare Fig 68 and 69). In the proximal tibiotarsus, of broiler fowls, sub-articular EVC networks extended

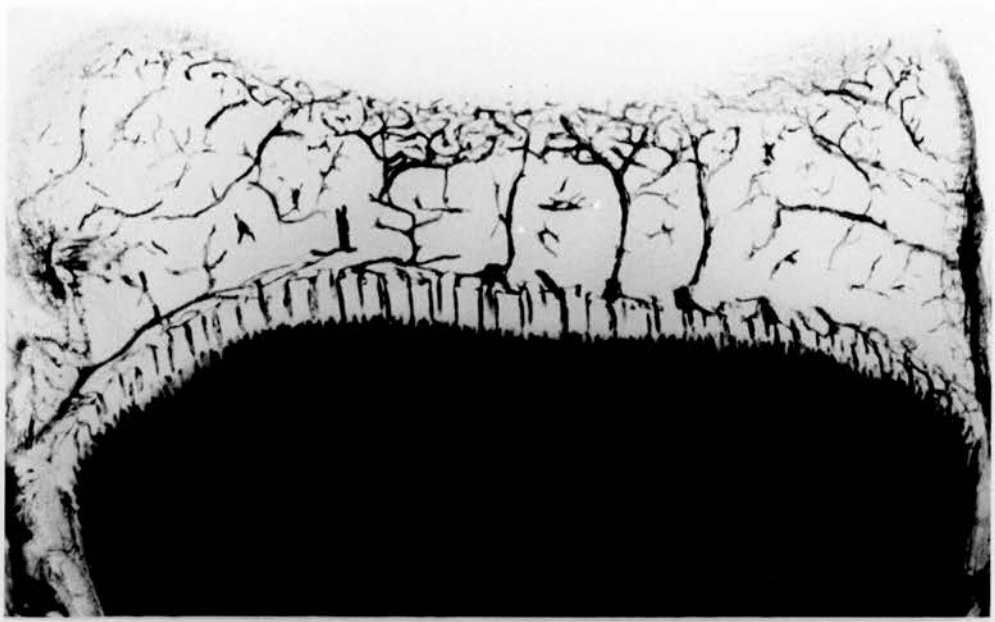


Fig 66. The distal tibiotarsus from a 6 week old broiler. There is a highly vascular subarticular network of EVCs. 1mm slab x10.

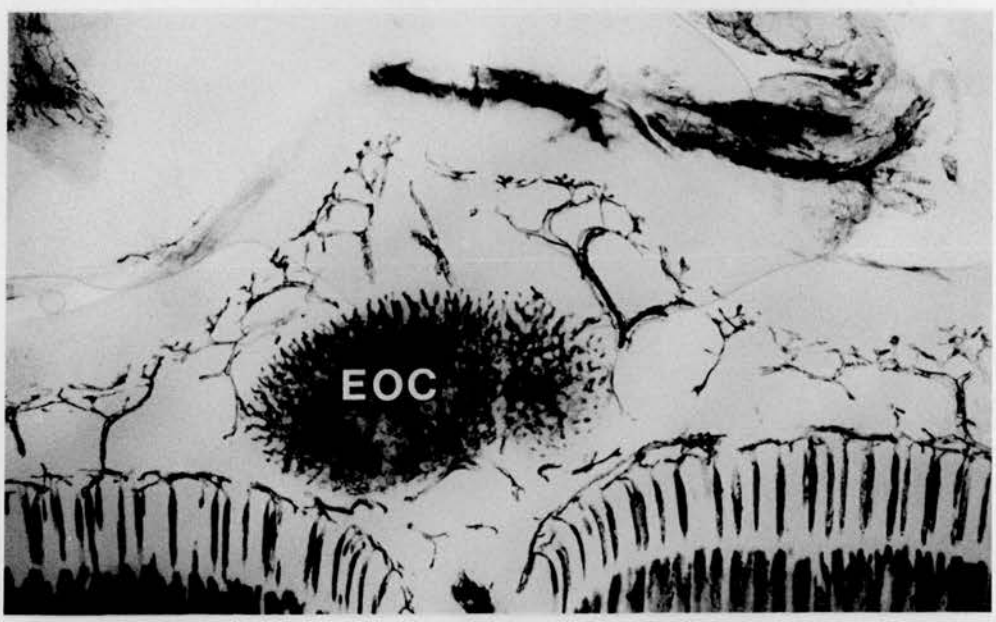


Fig 67. The proximal tarsometatarsus from a 3 week old broiler. The cartilaginous epiphysis is thicker than in the S line and there are many blind ending EVCs forming a subarticular plexus. 1mm slab x16.

into the subarticular cartilage of the condyles.

In broiler fowls at the point of insertion of the iliotrochanteric muscle into the lateral trochanter, the epiphyseal hyaline cartilage often appeared avascular (Fig 70). Adjacent to the avascular area, there were EVCs in the epiphyseal hyaline cartilage and also vessels in the tendon.

An accessory ossification centre occurred in the lateral epiphysis of the proximal tarsometatarsus in two broilers of six and eight weeks of age respectively (Fig 71). Ossification in the epiphyseal hyaline cartilage was occurring deep to the point of attachment of the lateral collateral ligament.

The vascular pattern of the bone extremities occurring during growth

The vascularity of the bone extremities in the pelvic limb of broiler fowls is described from hatching. Emphasis is placed on the variations from the normal S line pattern and abnormalities which occurred in the broilers.

Day old

The metaphyses all contained cartilage cores, which were perfused by transphyseal PEVs. Between specimens there was considerable variation in the number and extent of these elongated PEVs. Lateral branches sometimes arose from the transphyseal PEVs in the cartilaginous core. Occasional transphyseal PEVs would bifurcate (Fig 73).

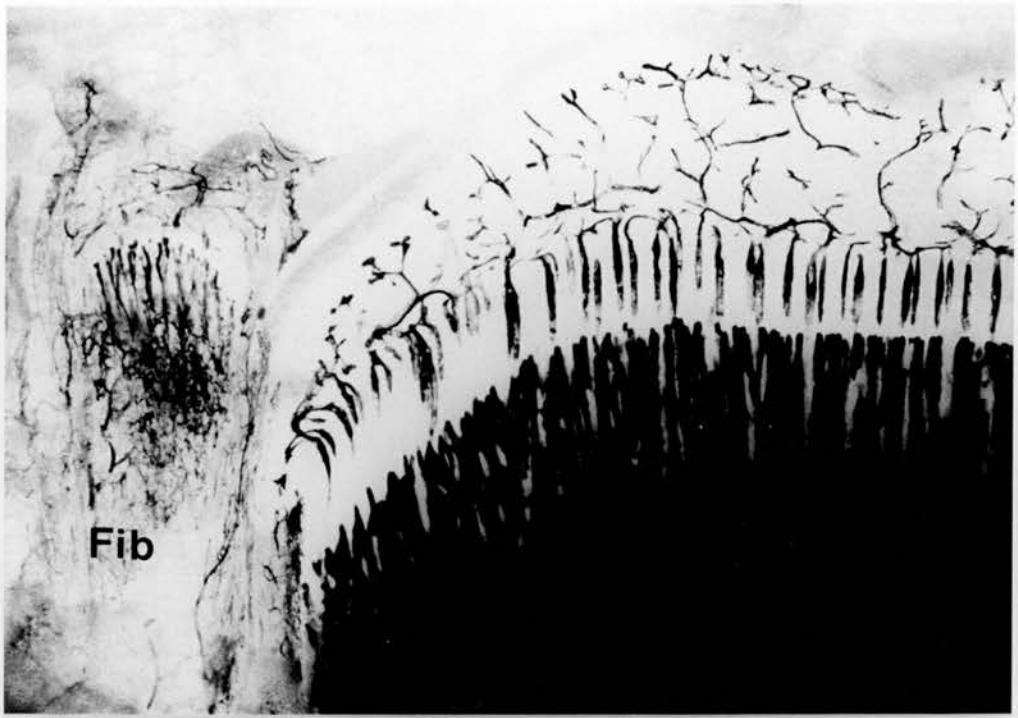


Fig 68. The lateral condyle of the proximal tibiotarsus and the fibula from a 4 week old broiler. There is a subarticular plexus of EVCs. The lateral physis is thickened and the PEVs do not penetrate to its full depth. 1mm slab x16.

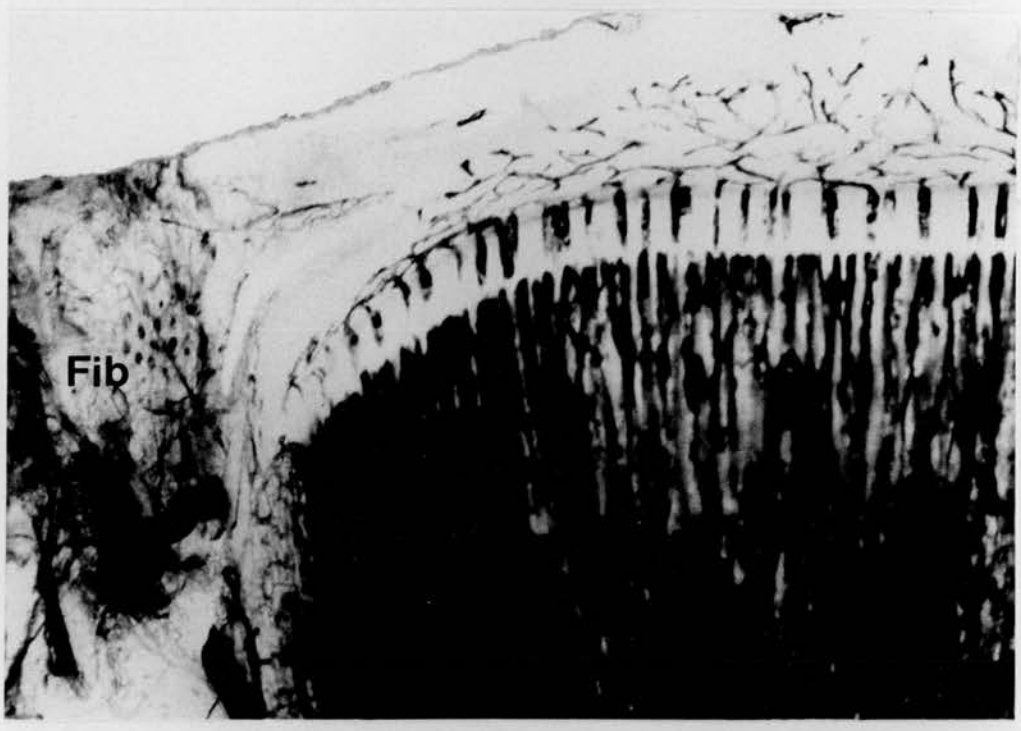


Fig 69. The lateral condyle and the fibula of a 4 week old S line. There are fewer subarticular EVCs and a more "normal" physis than the photomicrograph of the broiler in fig 68. 1mm slab x16.

Fig 70. The femoral trochanter from a 6 week old broiler. There is an avascular layer (arrowed) of cartilage deep to the insertion of the iliotrochanteric muscle. 1mm slab x16.

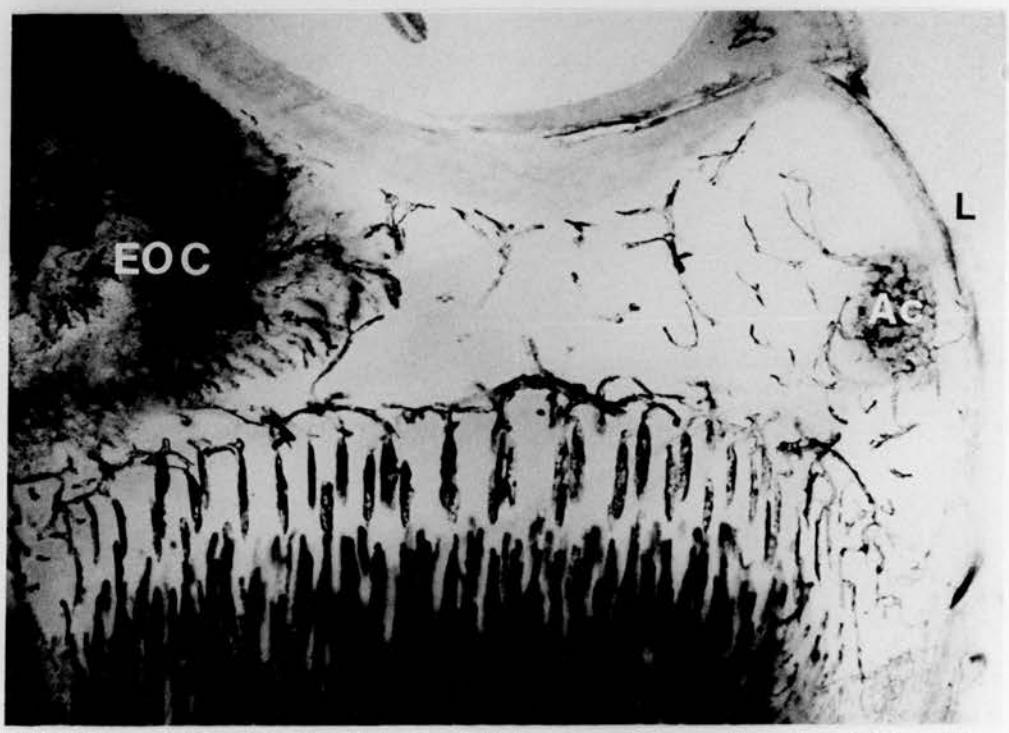


Fig 71. The proximal tarsometatarsus from a 8 week old broiler. An accesory ossification centre has formed deep to the point of attachment of the lateral collateral ligament. 1mm slab x20.

The central physis of some specimens contained few PEVs, especially in the larger bone extremities such as the proximal tibiotarsus. In one proximal femur there was a poor supply of MVs to the femoral head compared to the trochanter (Fig 74). The MVs were larger and wider than those in the S line fowls.

In most of the bone extremities the epiphyseal hyaline cartilage was highly vascular with many dividing EVCs which terminated as vascular buds. These were particularly concentrated in the thicker epiphyseal hyaline cartilage, such as in the femoral head and trochanter, distal femoral condyles, cnemial crests of the proximal tibiotarsus, distal tibiotarsal condyles and hypotarsus and intercondylar region of the proximal tarsometatarsus.

In some specimens the articular surface appeared uneven in contour. There were also, in some proximal femurs, fewer EVCs from the capital femoral ligament in broiler fowls and they supplied only a limited segment of the cartilaginous epiphysis.

Day two.

The cartilaginous epiphysis was well vascularised, and contained evenly spaced EVCs which supplied the physis with entire arrays of PEVs. In many specimens the size and spacing of the PEVs was uneven across the physeal cartilage. This was especially true at the centre of the physes. Transphyseal PEVs were present in the majority of metaphyses, but the PEVs of the proximal tarsometatarsi were restricted to the physes and were of normal size. In one extremity a PEV branched laterally (Fig 72) and then

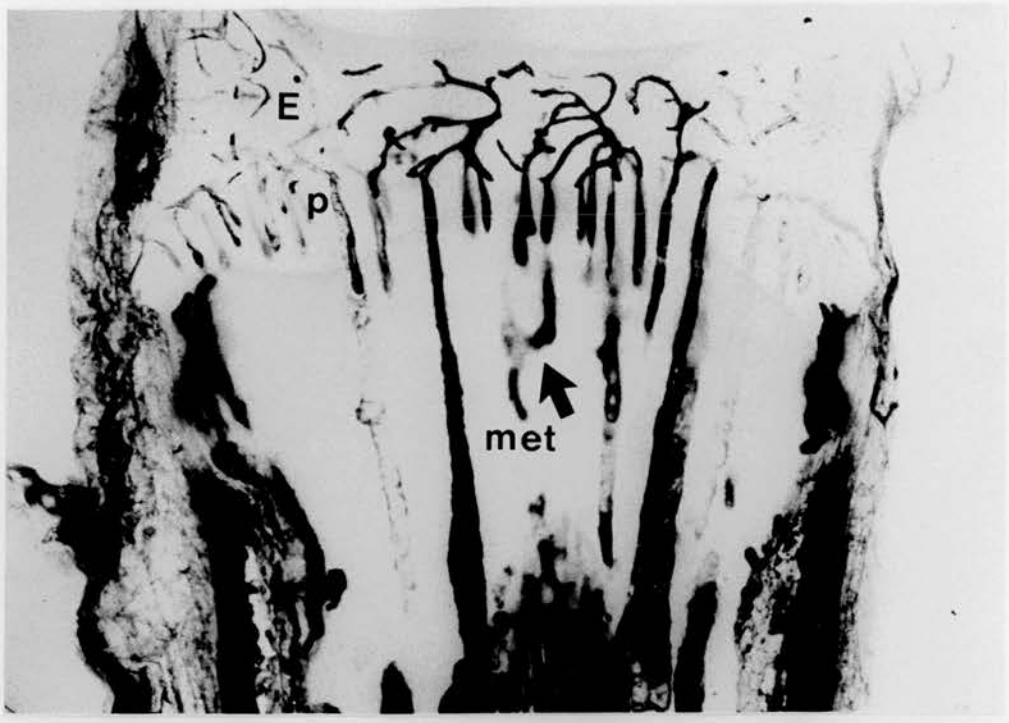


Fig 72. The distal tibiotarsus from a 2 day old broiler. MVs are only just approaching the periphery of the metaphysis. There are lateral branches (arrowed) from the long transphyseal PEVs. 1mm slab x25.

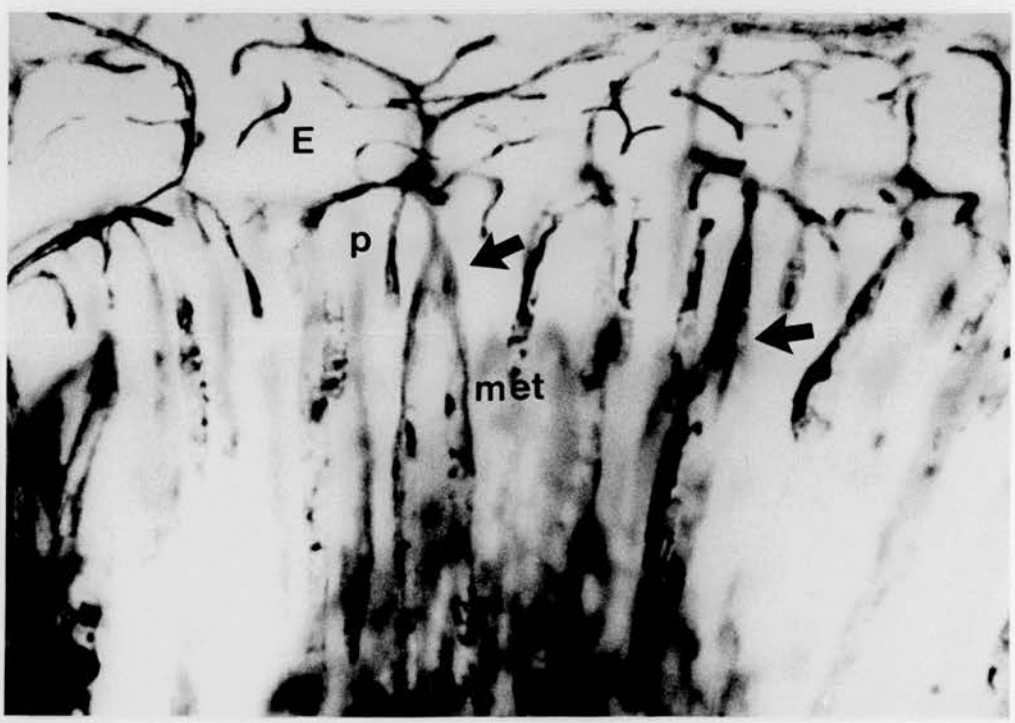


Fig 73. The proximal tibiotarsus from a day old broiler. Some of the transphyseal PEVs bifurcate (arrowed) as they penetrate the metaphysis. 1mm slab x40.

extend proximally and distally in the redundant canal left by an adjacent receding PEV. Metaphyseal cartilage cores were still present in the other four bone extremities. There was great variation between specimens in the rate of MV invasion. In some metaphyses there were irregular arrays of MVs. which were penetrated by transphyseal PEVs through the spaces between MVs (Fig 76). In others however there was still an extensive cartilage model in the centre of the metaphysis with long dividing transphyseal PEVs. especially in proximal tibiotarsi (Fig 81).

The extent of MV invasion of the cartilaginous metaphysis was minimal in some bone extremities. Frequently such specimens were poorly perfused by transphyseal PEVs. The advancing MVs, in these specimens. were blunt and appeared to be branching around rather than through the cartilage core (Fig 75). Lateral branching of MVs into the cartilage cone and then advancement along the redundant PEV canals was a feature of some specimens (Fig 79). In one proximal tibiotarsus there was vascular contact between an MV and a transphyseal PEV, with delayed MV invasion of the cartilage core.

Day five

In all cartilaginous epiphyses there were fully formed arrays of PEVs across physes. The PEV arrays were supplied by extensive systems of branching EVCs. In the metaphyses there was great variation in the extent to which MV arrays had formed. In some metaphyses there were complete, evenly spaced, arrays of MVs which formed a fully functional growth plate. In others there was an

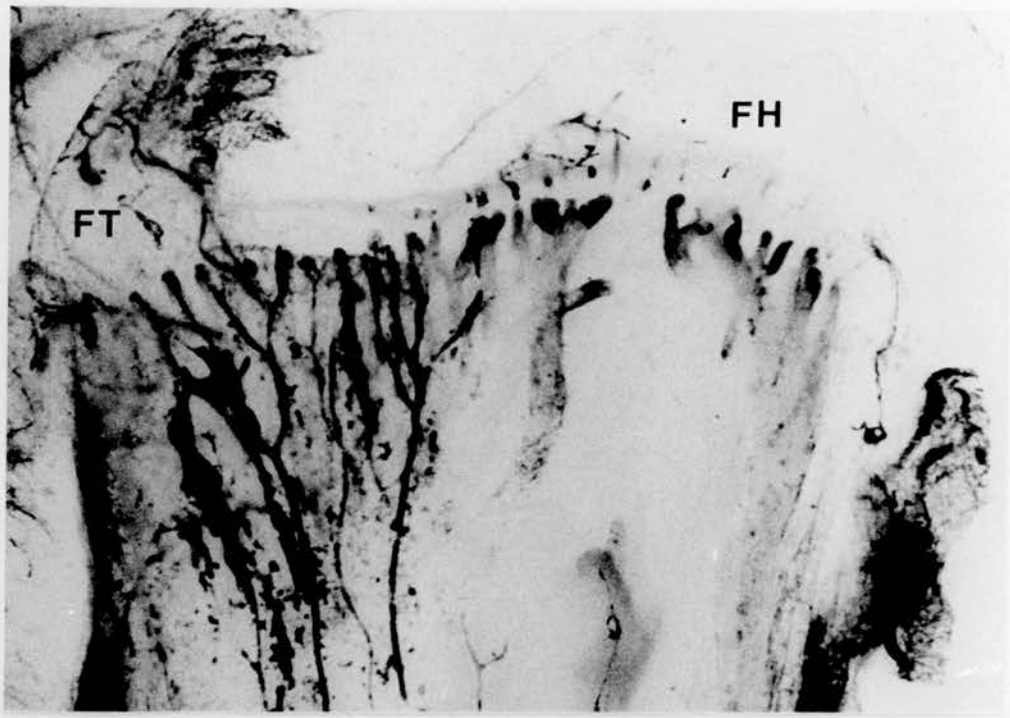


Fig 74. The proximal femur from a day old broiler. The femoral head is poorly supplied with MVs compared to the trochanter. 1mm slab x25.

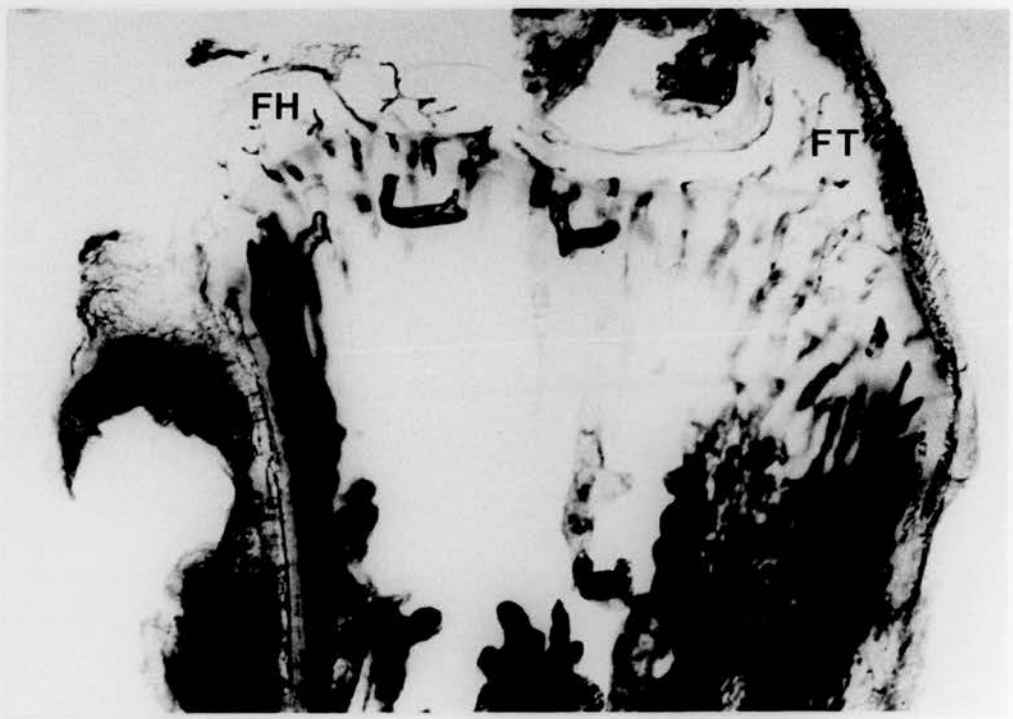


Fig 75. The proximal femur from a 2 day old broiler. There is disturbed invasion of the cartilaginous metaphysis by MVs. The MVs are blunt ending, widely spaced and have lateral branches. 1mm slab x25.

Fig 76. The distal tibiotarsus from a 2 day old broiler. The MVs are dividing to form an array across the metaphysis. There are long transphyseal PEVs crossing into the metaphyseal cartilage. 1mm slab x25.

Fig 77. The cnemial crest from the tibiotarsus of a 5 day old broiler. The PEV (arrowed) anastomoses with an MV. 1mm slab x 40.
(The insert is a histological section of the same area.MGT x40)

absence of MVs in the central metaphysis due to the presence of a cartilage core. In four of the proximal tibiotarsi there were transphyseal PEVs extending into this persistent cone of cartilage in the metaphysis, and frequently the transphyseal PEVs divided or branched in the retained cores of cartilage. In the centre of the condyles of the distal tibiotarsus there were frequently transphyseal PEVs which penetrated retained physeal cartilage.

In some bone extremities MVs were uneven both in size and the depth to which they penetrated the physis (Fig 78). The spacing between individual MVs was also unequal and irregular. These arrays were considered to be immature. There was in some physes direct vascular communication between PEVs and MVs (Fig 77).

In each pelvic limb of an individual, the bone extremities were usually at the same point of vascular development, but in some cases there was marked variation between the two limbs.

Dyschondroplasia was seen to occur in one proximal tarsometatarsus, the thickened lateral physis being due to a localized delay in MV invasion. The section of thickened physis was poorly perfused by PEVs but the MV arrays were fully developed below the lesion. A Polymaster slab was surface stained from this specimen (Fig 80).

Day seven.

In the majority of the bone extremities complete arrays of MVs were now present across metaphyses. Cartilage cores were still present in the central metaphyses of the proximal tibiotarsus and tarsometatarsus. Transphyseal PEVs crossed into

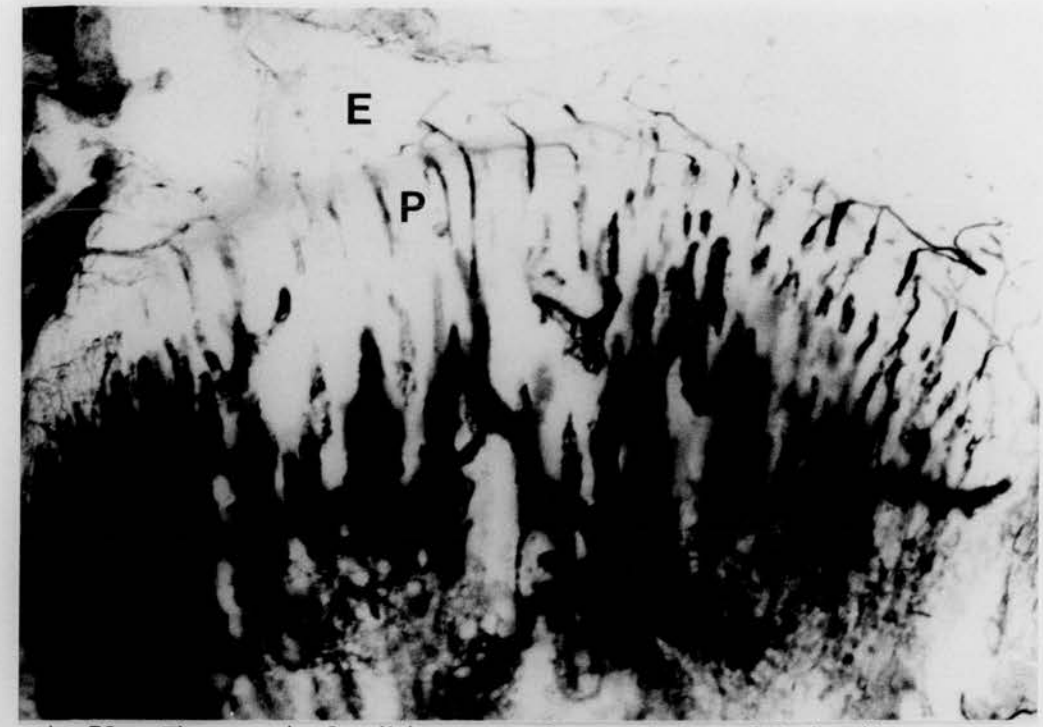


Fig 78. The proximal tibiotarsus from a 5 day old broiler. The MVs are " immature" (ie:They are uneven in depth of penetration of the physis, widely spaced and irregular in size). 1mm slab x25.

Fig 79. The distal tibiotarsus from a 2 day old broiler. The MV (arrowed) has branched laterally and is extending proximally and distally in an adjacent redundant PEV canal. 1mm slab x40.

the metaphyseal cartilage cone of the proximal tibiotarsus. The completed array of MVs in a proximal tibiotarsus were immature. In some proximal tibiotarsi there were localized increases in physeal width with PEV elongations.

Occasionally PEVs bifurcated in some of the physes, frequently in association with a marked local variation in PEV size. In specimens with bifurcating PEVs there was no evidence of disruption to endochondral ossification.

There was a marked variation in the size and length of PEVs in many physes. The contour of some PEVs was irregular due to lateral bulges. Other PEVs were also irregular in shape, forming a small sheet of vessels perpendicular to the direction of growth in the physis (Fig 82).

The trochanter of one proximal femur contained a region of PEV disruption. In the contralateral limb of the same bird, the cartilaginous epiphysis of the proximal fibula was avascular. There were budding EVCs entering and revascularising the avascular cartilage of this fibula (Fig 83).

Day nine

Retention of metaphyseal cartilage was now only seen in the proximal tibiotarsus, and in one case was associated with irregular MVs (Fig 84). There were abnormal PEVs in some proximal tibiotarsi. These aberrant PEVs were enlarged irregular and occasionally bifurcated. In some proximal tibiotarsi the MVs appeared elongated (Fig 85).

The cartilaginous epiphysis in the craniomedial aspect of the

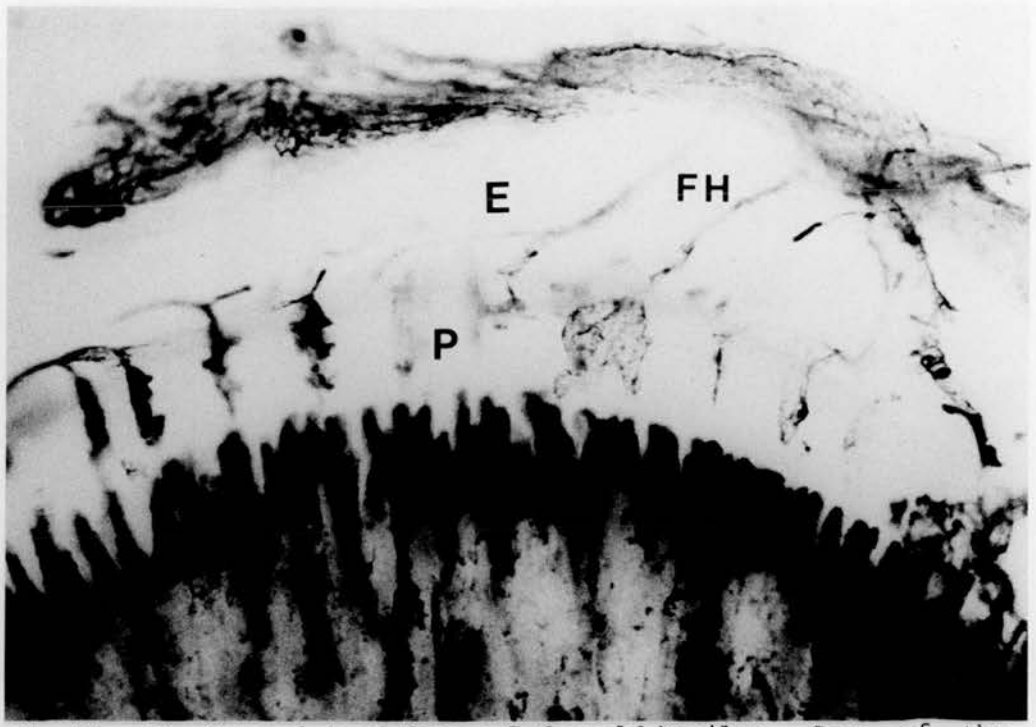


Fig 82. The femoral head from a 7 day old broiler. Some of the PEVs are grossly misshapen. The MVs are irregular in shape and change direction near the physis. 1mm slab x40.

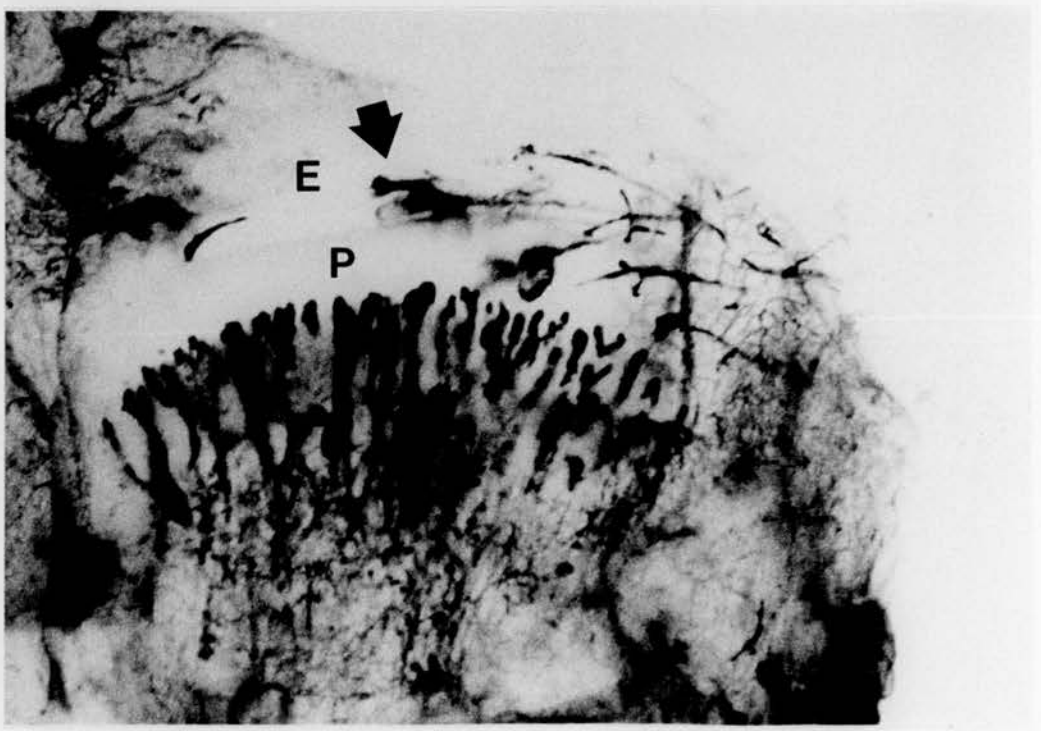


Fig 83. The proximal fibula from a 7 day old broiler. EVCs (arrowed) are penetrating an avascular area of the cartilaginous epiphysis. 1mm slab x25.

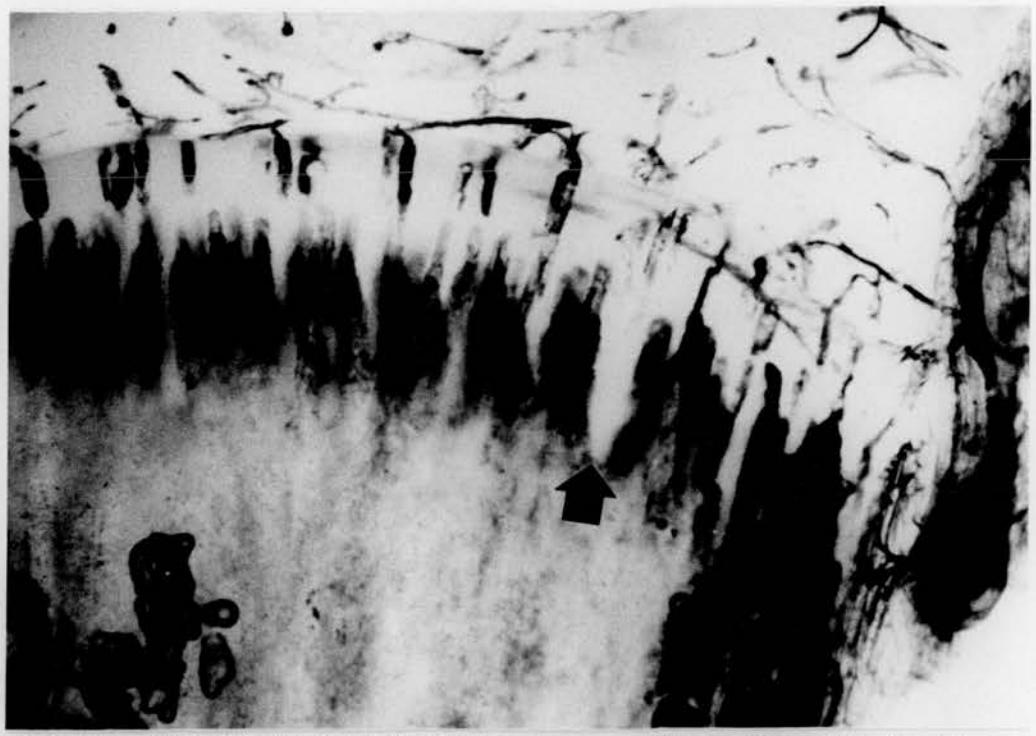


Fig 84. The proximal tibiotarsus from a nine day old broiler. The PEVs and MVs are irregular in shape with at some sites a wide gap between MVs (arrowed). 1mm slab x25.

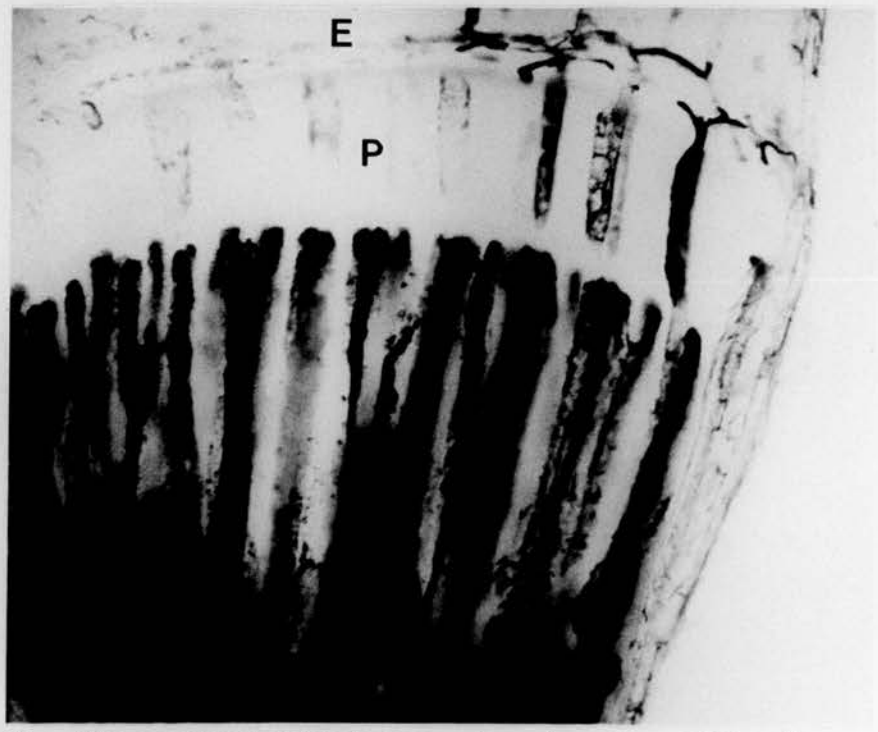


Fig 85. The proximal tibiotarsus of a nine day old broiler. The MVs are elongated. 1mm slab x40.

femoral head was poorly vascularised by EVCs in half of the specimens. The retinacular vessels surrounding the fovea of the femoral head were more extensive and covered a greater area than in the broiler fowls (Fig 86).

Day fourteen.

In half of the proximal femurs there was some degree of vascular disruption. The PEVs in the craniomedial femoral head were short and widely spaced, and they were supplied by EVCs from the medial retinacular vessels (Fig 87). In one specimen there was an absence of EVCs from the medial ICRVs, and the craniomedial femoral head was revascularising with EVCs derived from the capital femoral ligament (Fig 88). The physis of the mid-proximal femur was a common site of abnormal shaped PEVs and PEVs with transphyseal extensions or "tails" which appeared to make contact with MVs (Fig 89). These transphyseal tails were fine projections from the base of PEVs towards and into the metaphysis.

Elongation of PEVs predominantly occurred in areas of poor MV penetration of the physis. Thickening of the physis occurred in three of the distal femurs due to an increase in the depth of the prehypertrophic chondrocyte zone. The MVs were irregular and disrupted. There was lateral branching of MVs with delayed MV propogation into the physeal cartilage. Concurrently, medial bulging of the metaphyseal epicondyle occurred as MVs attempted to branch around the thickened physeal cartilage. Elongated PEVs penetrated the thickened physeal cartilage of some specimens.

The proximal tibiotarsi of 50% of specimens contained

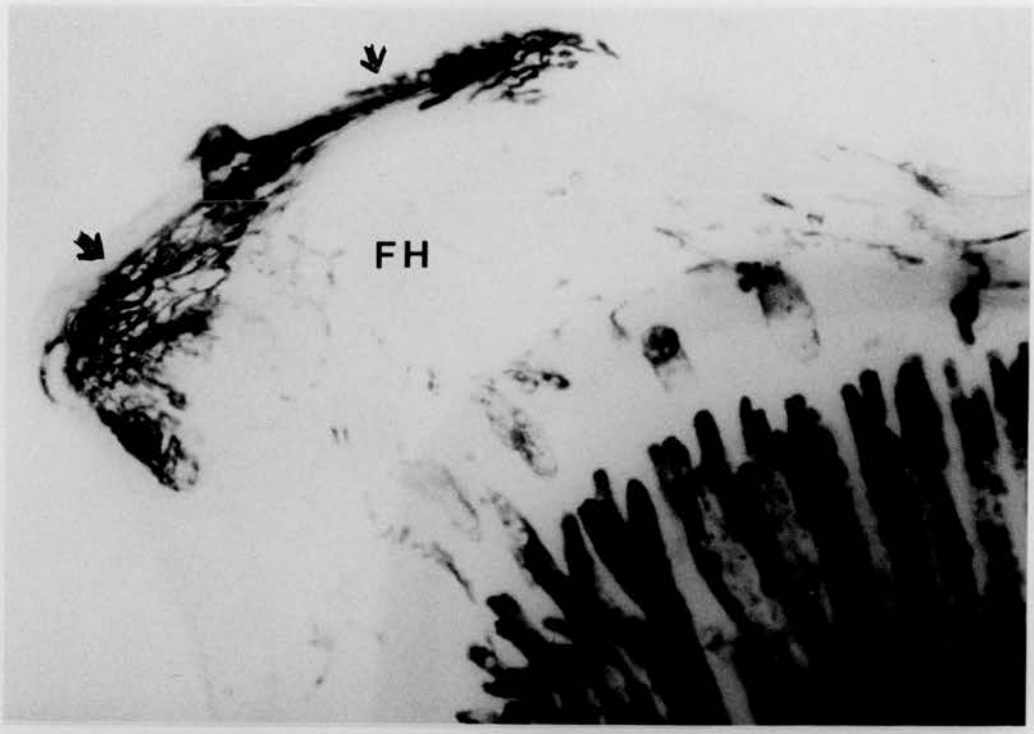


Fig 86. The femoral head from a nine day old broiler. The fovea (arrowed) is highly vascular. The PEVs are widely spaced. 1mm slab x40.

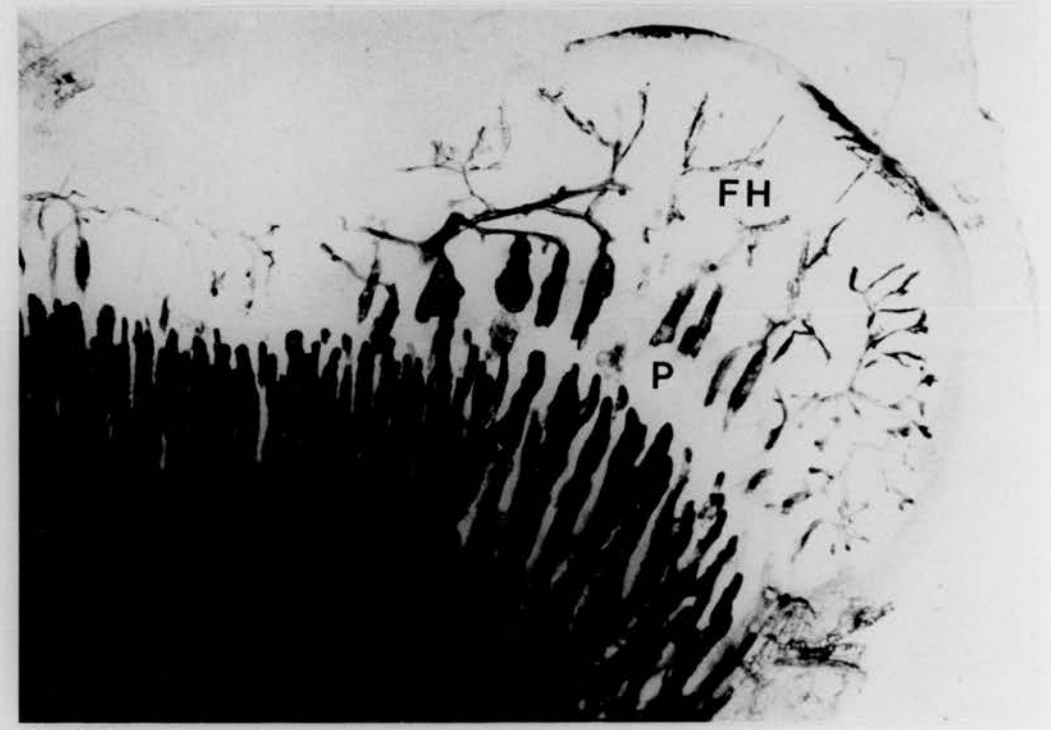


Fig 87. The femoral head from a 14 day old broiler. The PEVs vary markedly in size and in their depth of physeal penetration. 1mm slab x25

dyschondroplastic type lesions. These were all relatively minor lesions occurring at different sites in the physis. The most common site of physeal thickening however was in the tibiotarsus adjacent to the fibula. Typically there was delayed MV invasion of thickened physeal cartilage which was penetrated by elongated PEVs. PEVs occasionally bifurcated in the physis of the proximal tibiotarsus.

A typical dyschondroplastic lesion occurred in the fibula of one bird (Fig 90). The physis was thickened and MVs, although failing to penetrate the lesion, branched around its periphery. Elongated PEVs penetrated the periphery of the lesion, and lateral branches from these vessels extended into the thickened physeal cartilage. In this bird there were localized areas of disruption of MV invasion in most physes resulting in the frequent formation of dyschondroplastic lesions.

In the distal tibiotarsus the length of the PEVs was variable with some apparently extending through the physis into the metaphysis.

In most proximal tarsometatarsi there was a mild degree of either medial or lateral physeal thickening. This was, in most specimens, accompanied by elongation of some of the PEVs into the thickened cartilage (Fig 91).

Day 21.

The epiphyseal hyaline cartilage in this age group was highly vascular (Fig 95). There was a similar localized distribution of disrupted endochondral ossification to the previous age group.

Fig 90.Dyschondroplasia in the proximal fibula of a 14 day broiler.1mm slab x25.

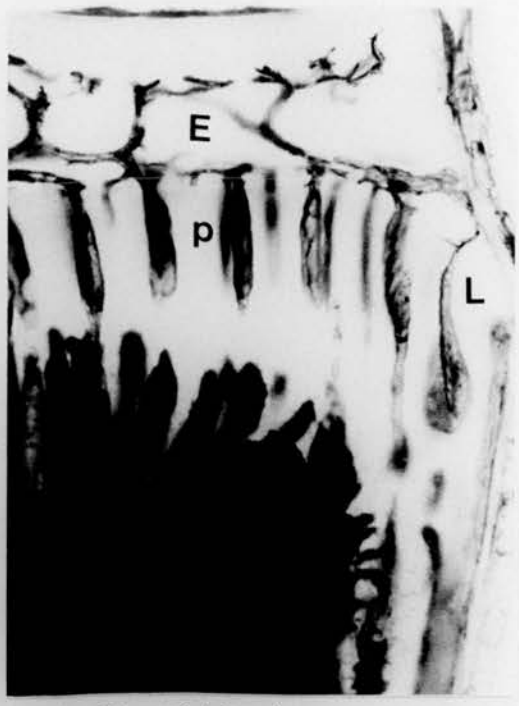


Fig 91. Thickening of the lateral physis in the tarsometatarsus of a 14 day old brioler.1mm slab x25.

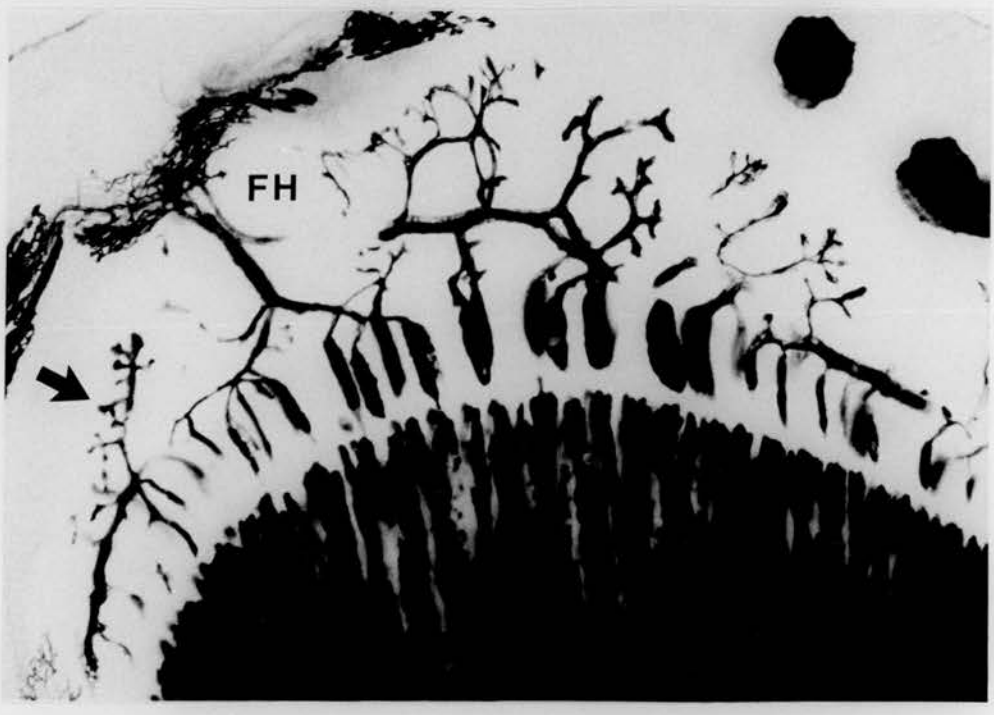


Fig 92. The femoral head of a 3 week old broiler. The cartilaginous epiphysis is highly vascular. The PEVs vary in size. There is subarticular plexus (arrowed) of EVCs and the fovea is highly vascular. 1mm slab x25.

In many of the bone extremities there was marked variation in the size of PEVs (Fig 92), although not necessarily associated with any obvious lesions.

An extensive cleft, containing haemorrhage was observed in the physeal cartilage of the femoral neck in one bird. The cleft took the form of a sheet of blue dye in the physis perpendicular to the direction of growth and transecting the PEVs. The PEVs appeared grossly intact and their perfusion had not been disrupted by the lesion. In one proximal tarsometatarsus there was a change in angulation of all the MVs at a point equidistant from the epiphyseal/physeal junction (Fig 93). The effect was similar to fronds of seaweed being aligned underwater by the current.

Amalgamation of two adjacent PEVs occurred in the distal tibiotarsus of one specimen (Fig 94).

Day 28.

A similar range and frequency of non uniform physeal vascularity and lesions occurred in this age group. The PEVs frequently had transphyseal tails. small lateral bulges or other irregularities in shape (Fig 96). A sheet of blue dye, parallel to the direction of growth. suggestive of haemorrhage was present in the physeal cartilage of one proximal femur (Fig 97). This lesion was identical to the one occurring in the previous age group. Histological sections demonstrated red blood cells and cellular debris in this cleft.

The network of retinacular vessels surrounding the fovea were prominent in broiler fowls. The vessels extended on to the

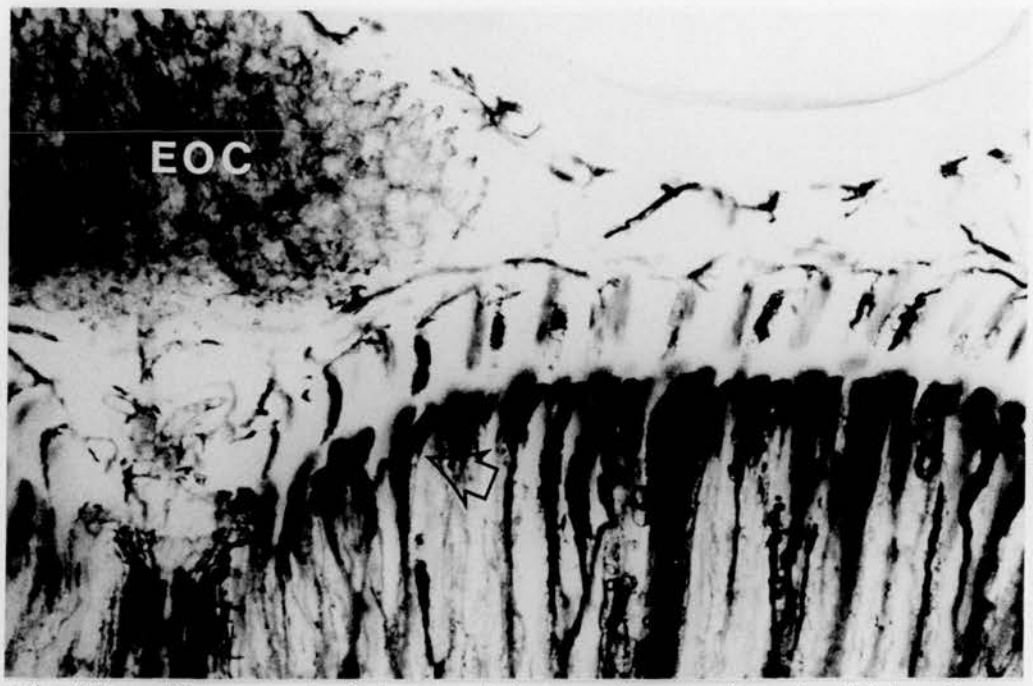


Fig 93. The proximal tarsometatarsus from a 3 week old broiler. There is a wave of changed angulation (arrowed) in the MVs. 1mm slab x25.

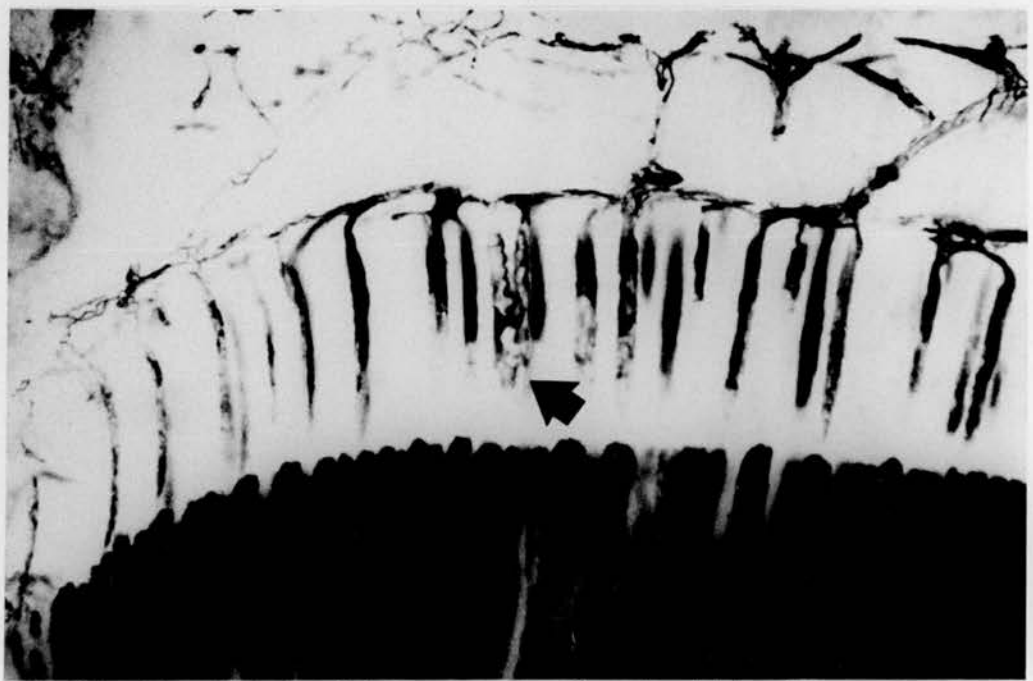


Fig 94. The distal tibiotarsus of a 3 week old broiler. Two PEVs are conjoined (arrowed). 1mm slab x25.

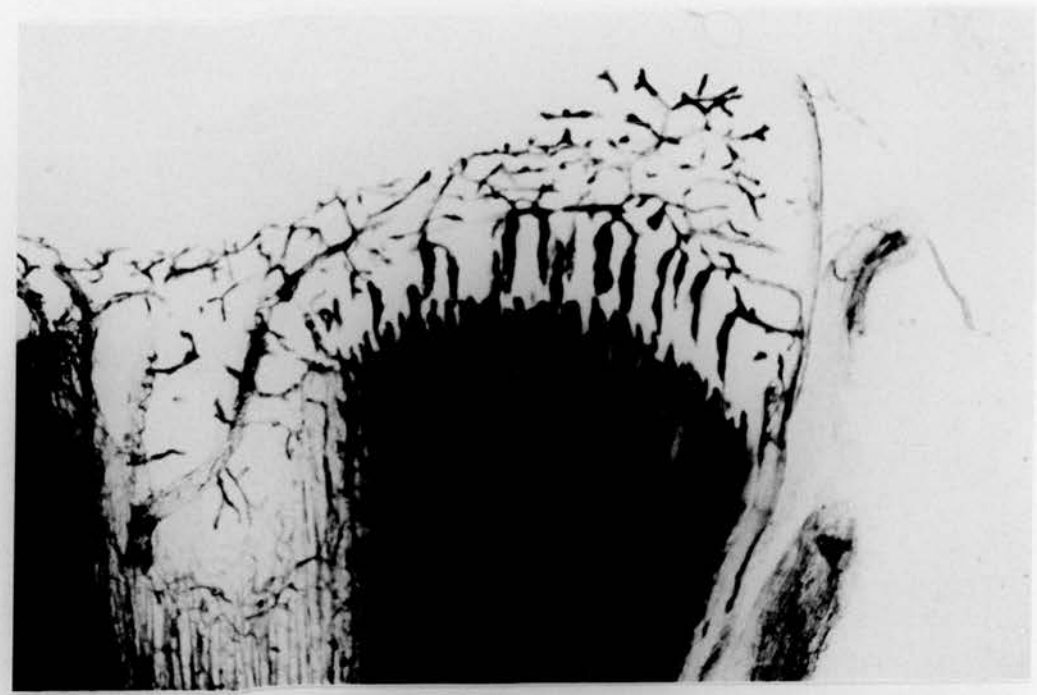


Fig 95. The distal femur from a 3 week old broiler. The epiphysis is highly vascular. There are EVC networks in the cartilaginous epiphysis. 1mm slab x16.

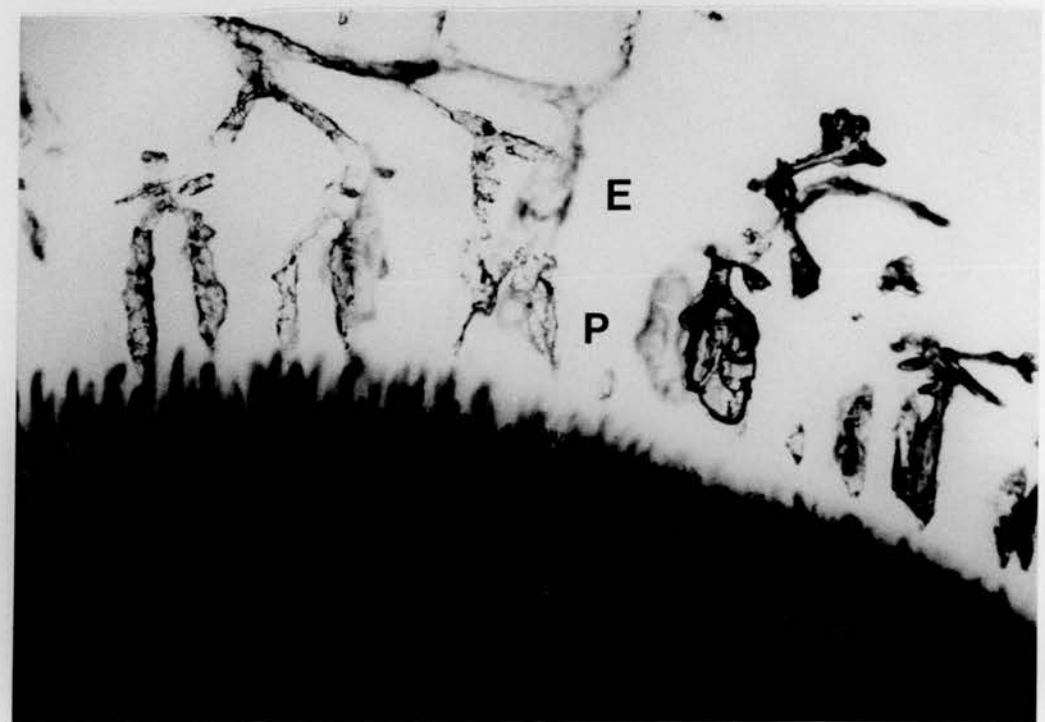


Fig 96. The femoral head of a 4 week old broiler. The PEVs are irregular in shape. 1mm slab x40.

surface of the articular cartilage of the femoral head.

The physeal cartilage in the lateral tibiotarsus adjacent to the fibula was frequently thickened (Fig 68). MV invasion was delayed and small dyschondroplastic lesions occurred at this site.

Day 42.

In the cartilaginous epiphyses of three proximal femurs there were avascular regions undergoing revascularisation. Two of the lesions occurred in the craniomedial femoral head and the third in the femoral trochanter. Revascularisation of the lesions in the femoral heads was from capital femoral ligament derived EVCs.

Clefts containing haemorrhage were present in the physis of a femoral head, a proximal tibiotarsus and a proximal tarsometatarsus (Fig 98). In the proximal tarsometatarsus, of another bird, irregular lateral bulges in the PEVs were present equidistant from the cartilaginous epiphysis (Fig 100). The MVs were irregular in length and spacing. In one proximal femur there was an avascular core of cartilage in the metaphysis of the trochanter (Fig 99).

The contour created by the periperal vessels in the enlarging EOCs in the broiler fowl was uneven (Fig 101 and 102).

Day 56.

The physeal cartilage in two of the femoral heads was thickened with poor MV penetration. Marked variation in the size of PEVs was seen in most slabs of proximal femurs and the occasional bifurcating PEV was seen. In the cranial femoral

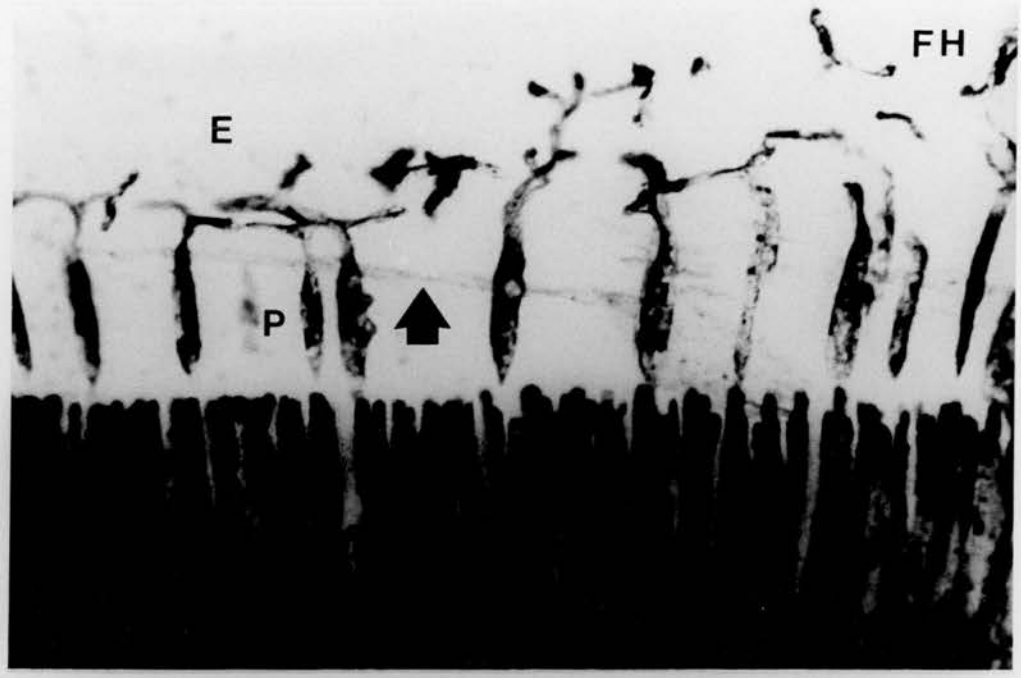


Fig 97. The femoral neck of a 4 week old broiler. There is a sheet of haemorrhage (arrowed) in the physis. 1mm slab x40.

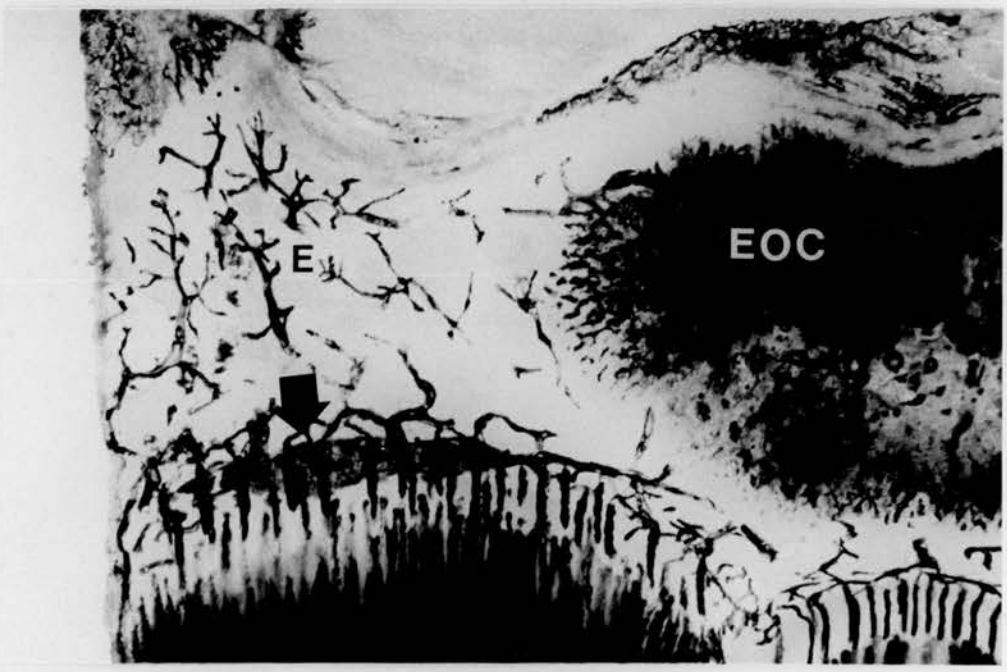


Fig 98. The proximal tarsometatarsus of a 6 week old broiler. The cartilaginous epiphysis is misshapen. There is a sheet of haemorrhage in the physis (arrowed). 1mm slab x16.

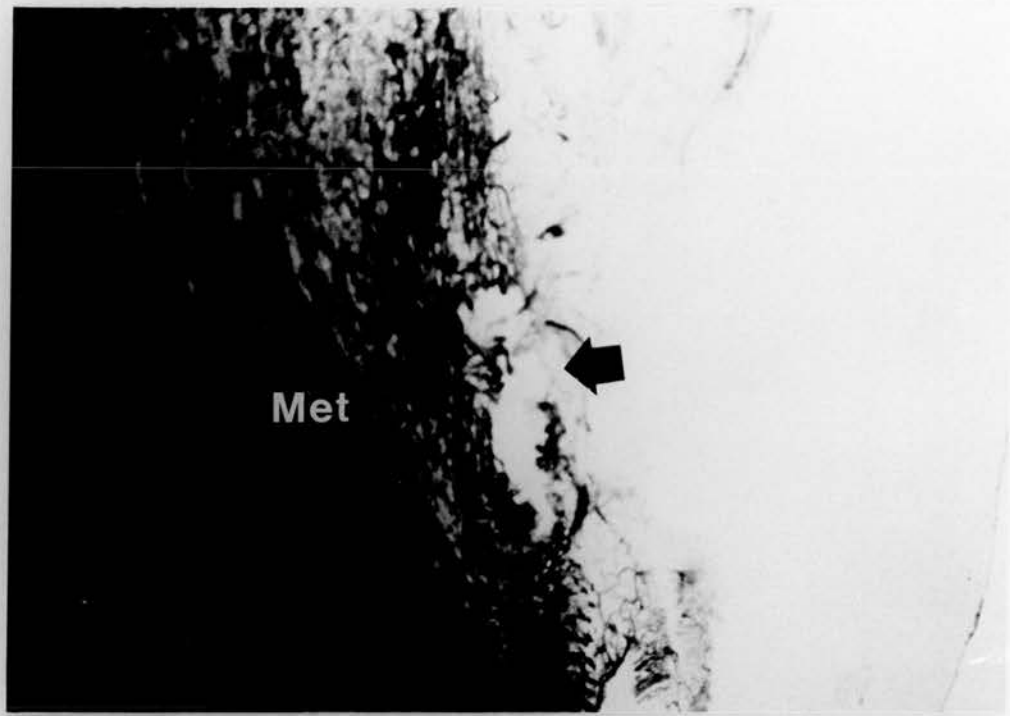


Fig 99. The femoral trochanter of a 6 week old broiler. There is an avascular defect (arrowed), in the metaphysis, surrounded by MVs. 1mm slab x20.

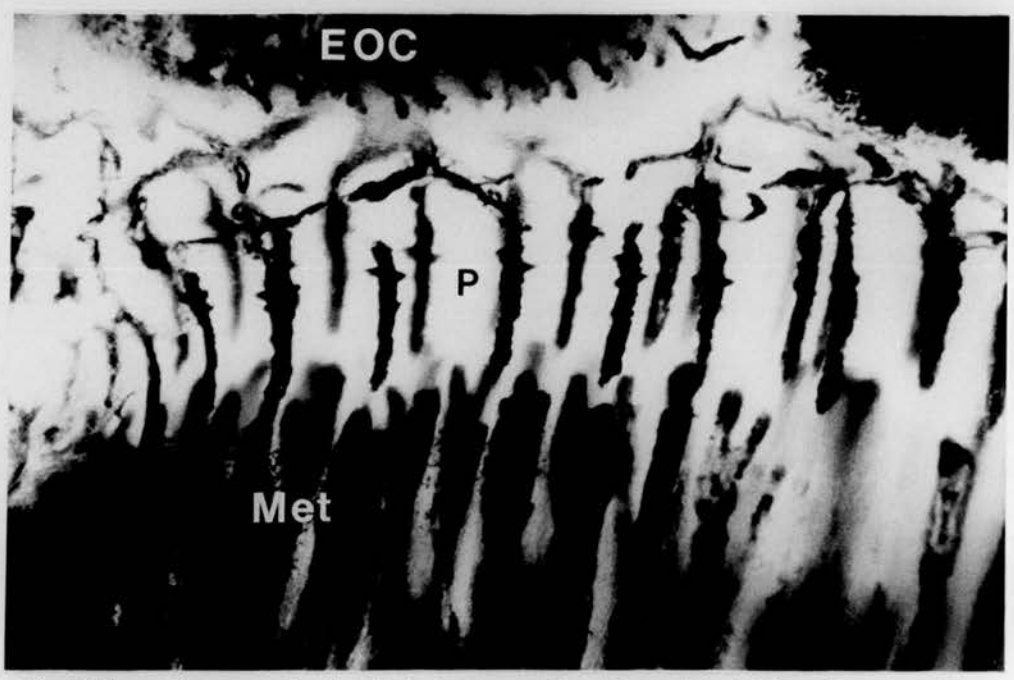


Fig 100. The proximal tibiotarsus of a 6 week old broiler. There are lateral bulges in many of the PEVs equidistant from the metaphysis. 1mm slab x40.

trochanter of one bird, delayed MV invasion and marked physeal thickening was associated with occluded PEVs and EVCs. Advancing EVCs were revascularising the cartilaginous epiphysis, and formed a classic "starburst" of vessels descending into the avascular cartilage.

In the distal femoral condyles there was frequent flattening of the normal contours created by the MV arrays.

Dyschondroplastic defects were present in 25% of the proximal tibiotarsi. The severity of the lesions varied from a slight localized thickening of the physis (Fig 103) to a large mass of retained physeal cartilage (Fig 104) which occupied the metaphysis. Occasionally a generalised thickening of the physis occurred. A common feature of all the lesions was the disarray of MVs below abnormal physeal cartilage. MVs were widened and there was an overall reduction in their numbers. Branches from MVs attempted to advance around the lesions.

The vascularity of the distal tibiotarsi was apparently normal in all the specimens of this age examined. In the proximal tarsometatarsi there were occasionally localized areas of slight physeal thickening. There was a cleft containing haemorrhage in the physis of one proximal tarsometatarsus.

Day 70.

The PEVs in the distal femur were now widely spaced and considerably shortened in length.

Small areas of delayed MV invasion below thickened physeal cartilage were present in two proximal tibiotarsi and one proximal

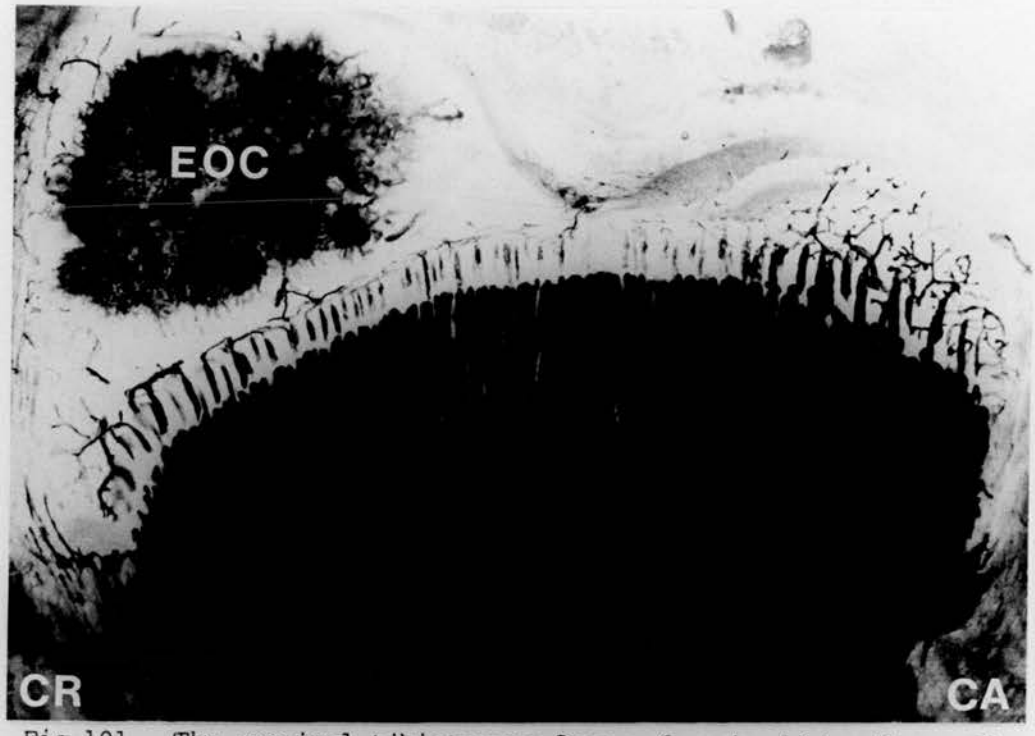


Fig 101. The proximal tibiotarsus from a 6 week old broiler. The periphery of the EOC is uneven in contour. The size of the PEVs varies across the physis. 1mm slab x10.

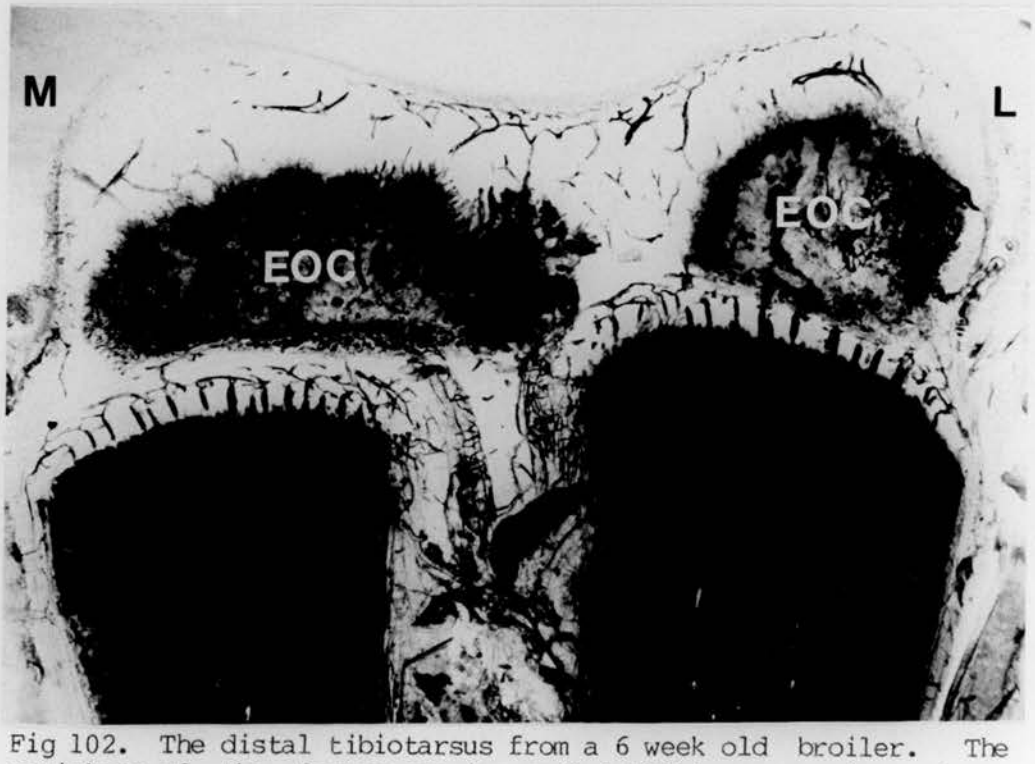


Fig 102. The distal tibiotarsus from a 6 week old broiler. The periphery of the faster growing medial EOC is uneven in contour. 1mm slab x10.

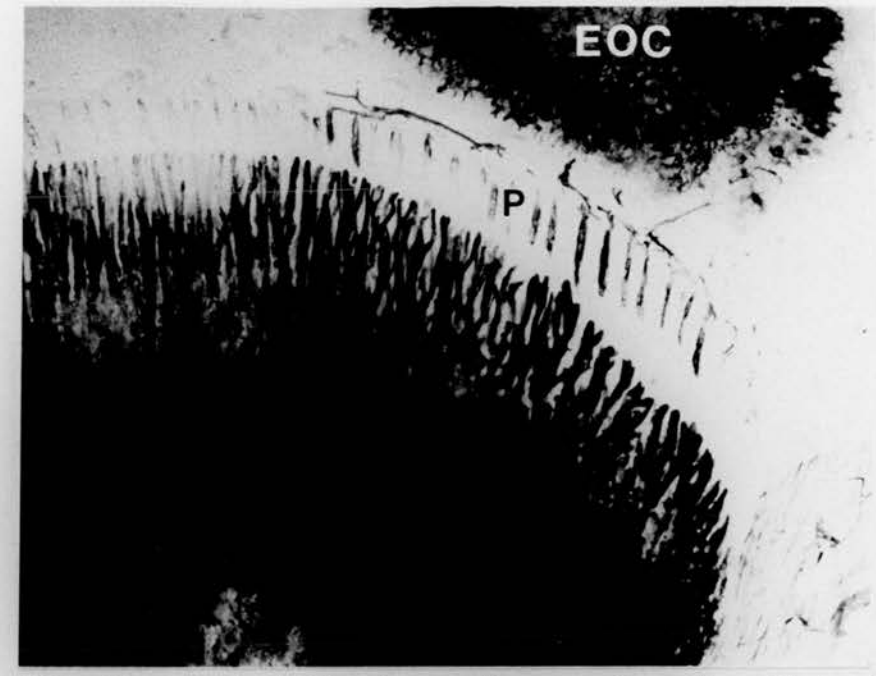


Fig 103. The proximal tibiotarsus from a 6 week old broiler. There is thickening of the physis below the EOC. The MVs beneath the thickened physis are irregular in shape, enlarged and blunt ending. 1mm slab x16.

Fig 104. The proximal tibiotarsus of an 8 week old broiler. The metaphysis is occupied by a large dyschondroplastic defect (D). MVs are penetrating the periphery of the defect. The PEVs are either normal or slightly elongated. 1mm slab x10.

femur.

In some areas of physeal thickening the only apparent vascular disturbance was a delay in, and disruption of MV invasion. PEVs were present and were either normal or elongated. In other examples of physeal thickening there was occlusion of PEVs and EVCs. and such lesions were being revascularised by EVCs budding into the avascular cartilage. Occasional bifurcating PEV were a feature of most physes. Transphyseal PEVs when present most frequently penetrated the physis between widely spaced MVs. In one tibiotarsus a large PEV which flared, crossed into the metaphysis and anastomosed with the MVs (Fig 105). In one dyschondroplastic type lesion in the femoral head there was occlusion of EVCs and PEVs (Fig 106). This area was being revascularised by EVCs from the capital femoral ligament. In one proximal tibiotarsus a cleft containing haemorrhage was found in the physis below the EOC (Fig 107). In a distal tibiotarsus deep to the site of attachment of the collateral ligament there was avascular epiphyseal hyaline cartilage (Fig 108).

Day 105.

In all the specimens the EVCs were disappearing from the epiphyseal hyaline cartilage into which the MVs were slowly ingressing. The rate of progression of the MVs into the epiphyseal hyaline cartilage was variable and non uniform. The ossified metaphysis was uneven in profile especially in the femoral head.

In this age group of broiler fowls two different types of

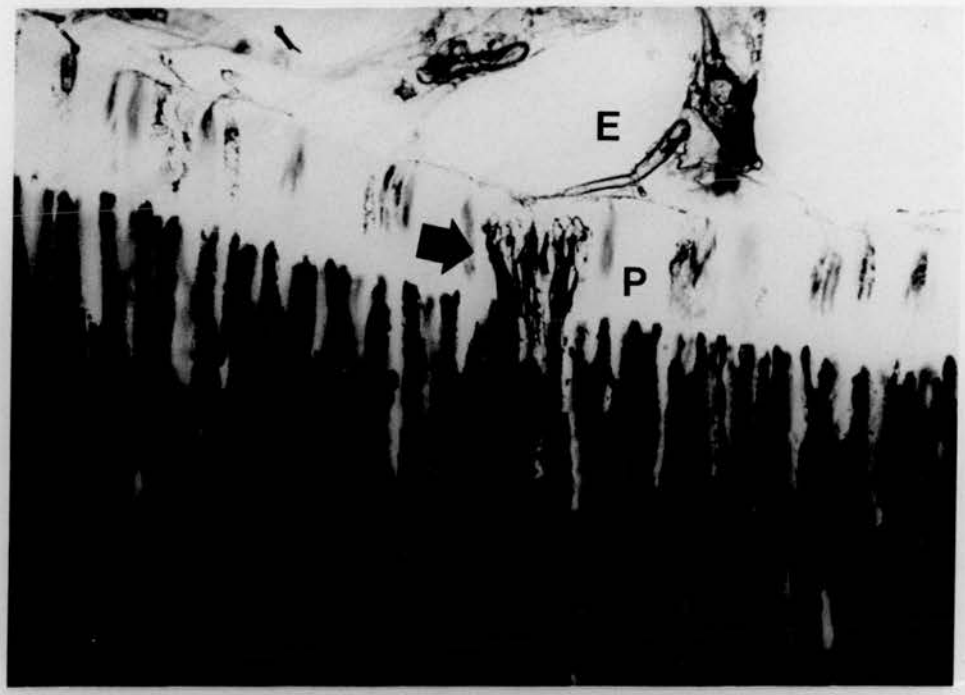


Fig 105. The proximal tibiotarsus from a 10 week old broiler. There is an enlarged flaring PEV (arrowed) which crosses the physis to anastomose with the MVs. 1mm slab x30.

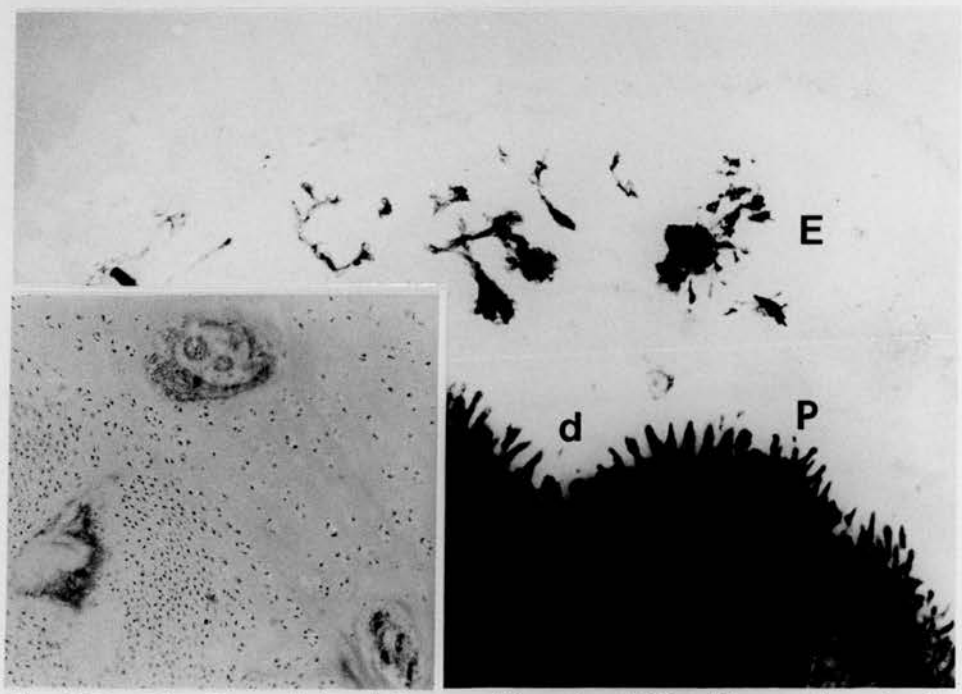


Fig 106. The femoral head from a 10 week old broiler. The physis is thickened. There is a mass of avascular cartilage in the metaphysis (d). The EVCs above the avascular cartilage are revascularising the cartilaginous epiphysis. 1mm slab x16.
(Insert: An occluded EVC in the avascular cartilaginous epiphysis.MGT X50.)

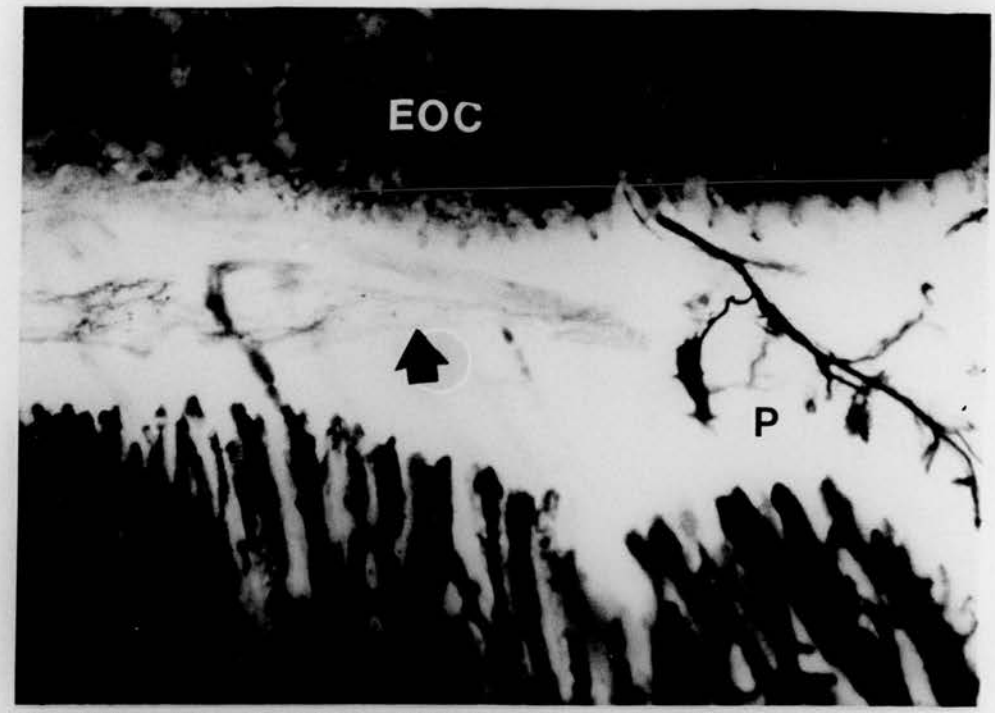


Fig 107. The proximal tibiotarsus from a 10 week old broiler. The physis below the EOC is thickened and contains a sheet of haemorrhage (arrowed). PEVs are absent and MVs are widely spaced and irregular at the site of the lesion. 1mm slab x40.

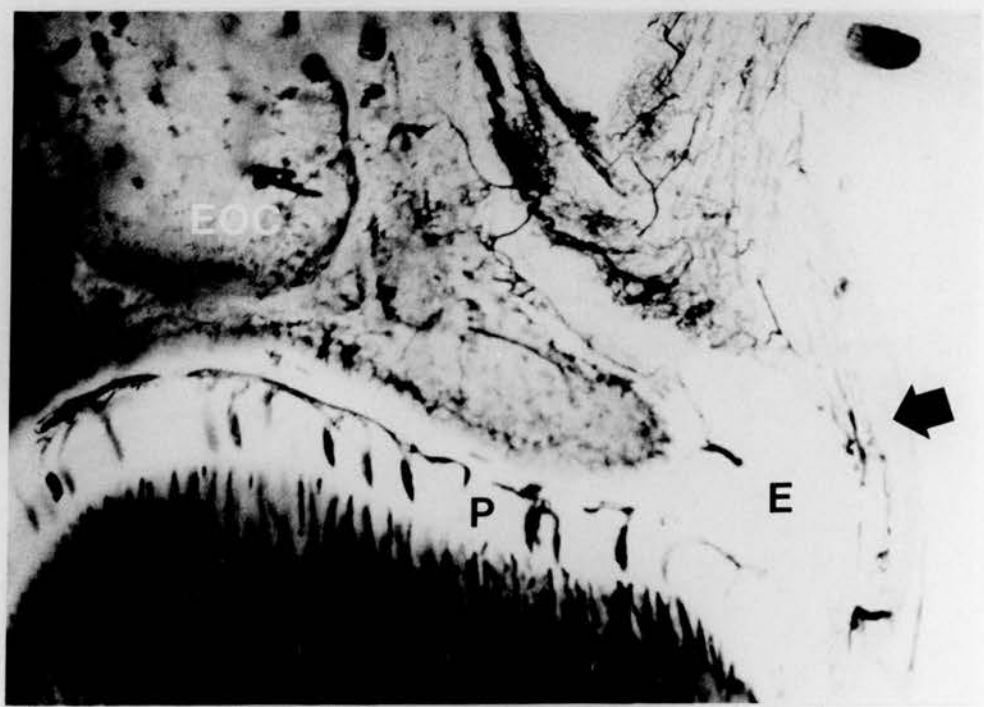


Fig 108. The distal tibiotarsus from a 10 week old broiler. The cartilaginous epiphysis which underlies the point of attachment of the lateral collateral ligament is avascular (arrowed). 1mm slab x20.

lesion commonly occurred. They both involved the retention of avascular epiphyseal hyaline cartilage; the first in the epiphysis and the other in the metaphysis.

1) Delayed MV erosion of the epiphyseal hyaline cartilage. This was observed in the lateral femoral trochanter with cartilage retention deep to the attachment of the iliotrochanteric muscle (Fig 109).

2) Retained epiphyseal hyaline cartilage was found to occur in the metaphysis of the distal femur and distal tibiotarsus deep to the point of attachment of the collateral ligaments (Fig 110 and 111).

An extension of the metaphysis formed a tongue (plate) at the site of ligament or tendon attachment (Fig 111).

Day 140

The process of ossification of the epiphyseal hyaline cartilage was very variable with many of the epiphyses still containing large quantities of hyaline cartilage (Fig 112). The remnants of hyaline cartilage were located around the periphery of the old cartilaginous epiphysis and adjacent to the articular cartilage. The majority of cartilage remnants were avascular.

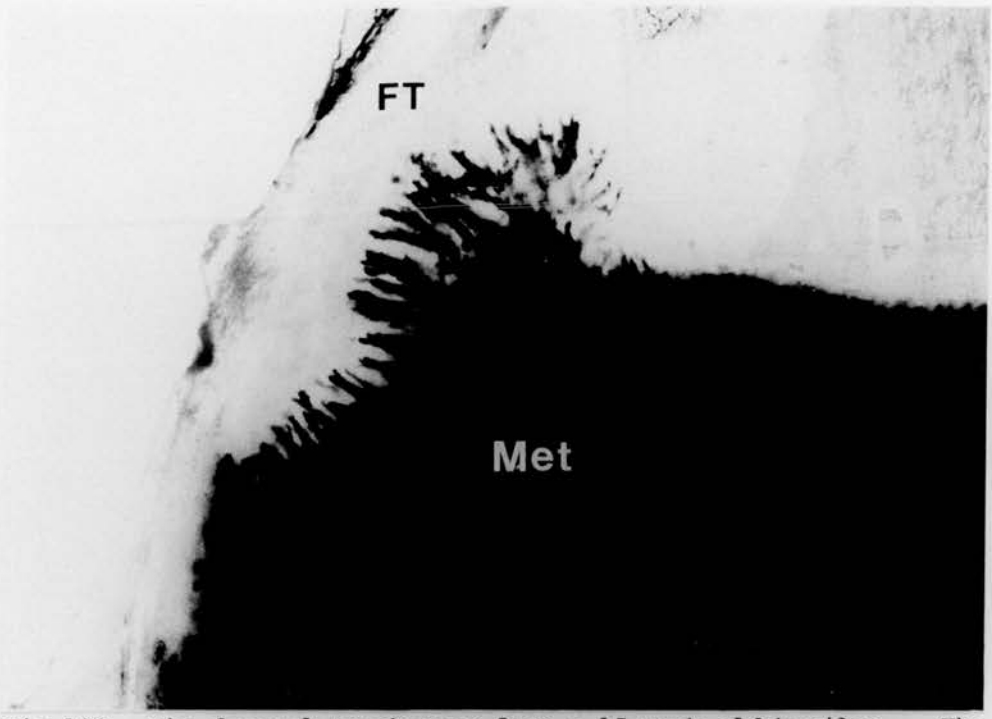


Fig 109. The femoral trochanter from a 15 week old broiler. The avascular cartilaginous epiphysis is slowly being eroded by MVs which are irregular and elongated. 1mm slab x15.

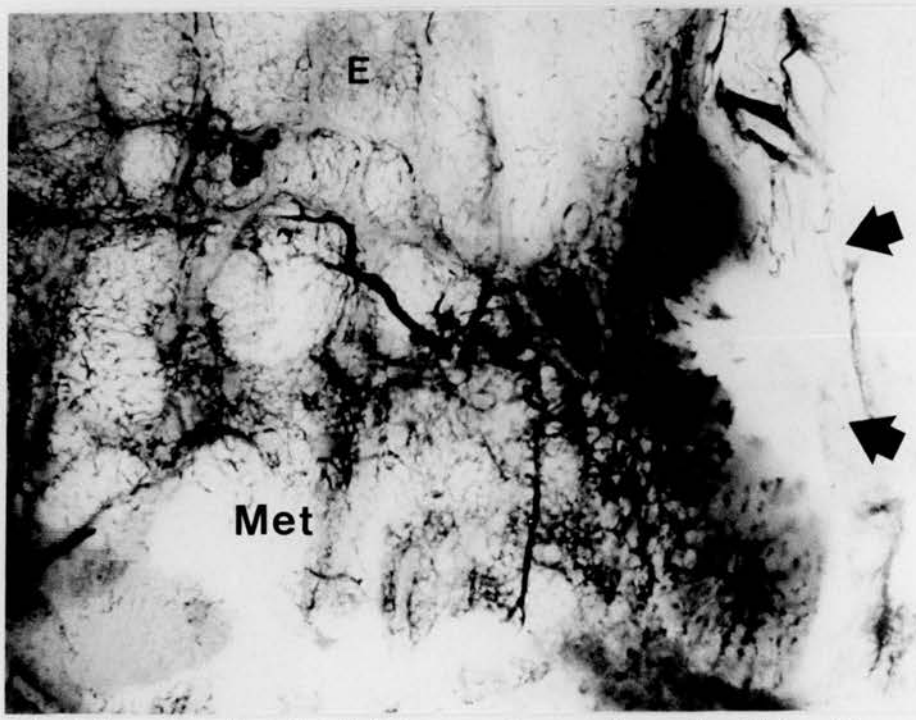


Fig 110. The distal tibiotarsus from a 15 week old broiler. Most of the cartilaginous epiphysis is now ossified. Hyaline cartilage is still present at the point of attachment of the collateral ligament (arrowed). 1mm slab x20.

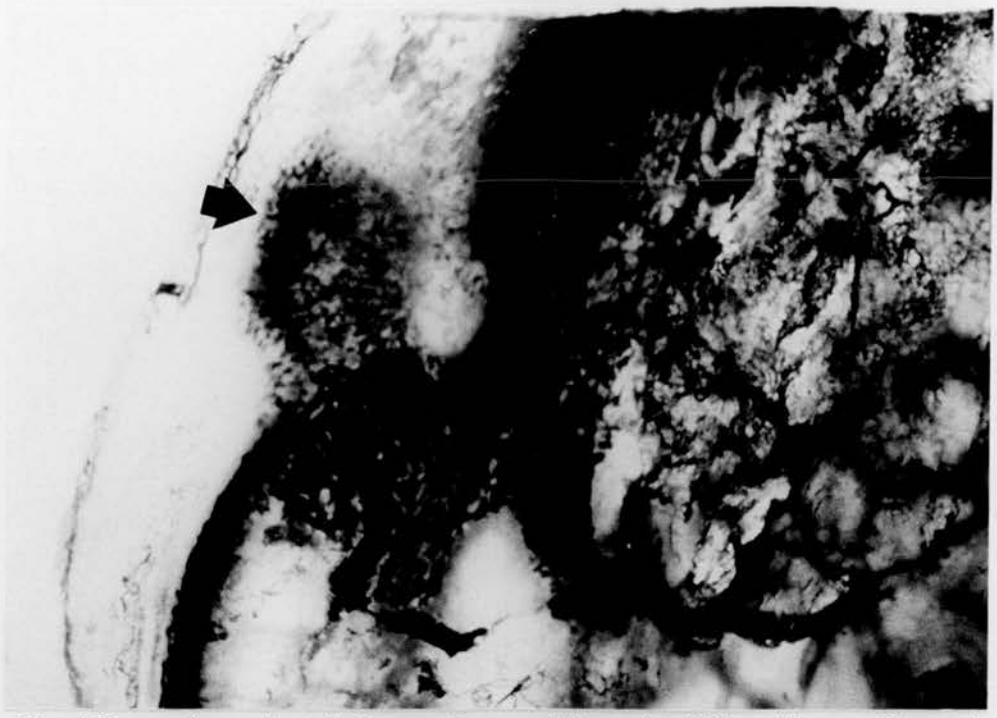


Fig 111. The distal femur from a 15 week old broiler. There is the formation of a plate of metaphyseal bone (arrowed), in the cartilaginous remnants of the epiphysis, deep to the attachment of the collateral ligament. 1mm slab x16.

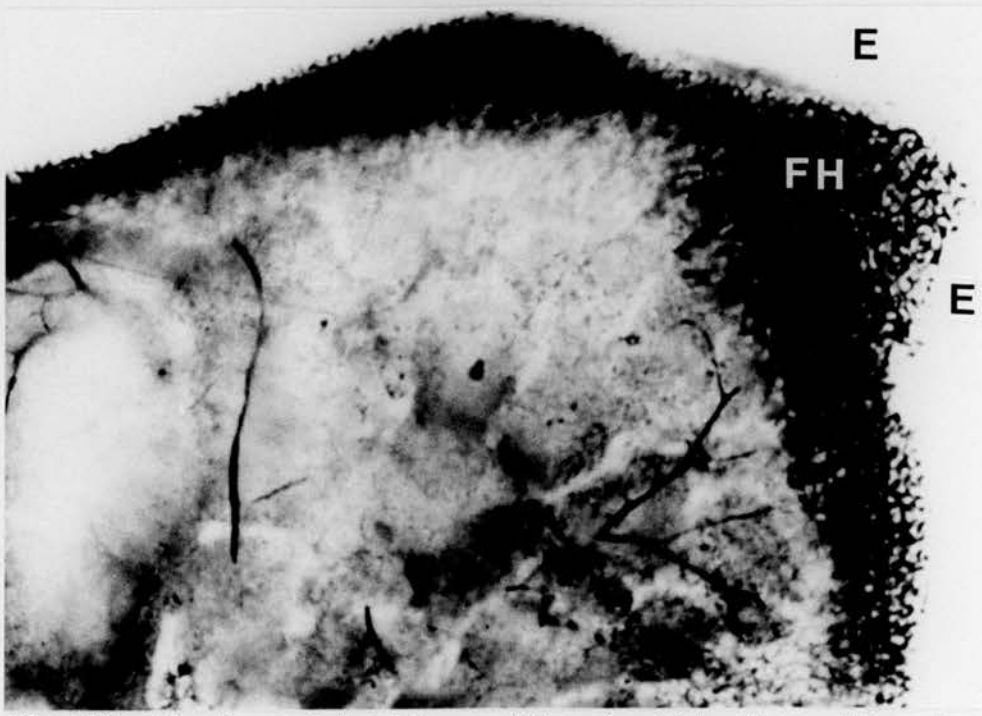


Fig 112. The femoral head from a 20 week old broiler. There is a delay in the rate of MV invasion of the avascular cartilaginous epiphysis. 1mm slab x10.

DISCUSSION

The pattern of growth and weight gain in the broiler fowl was very different from the S line fowl. The difference between the average male and female weights was evident at a younger age in the S line. The weight at hatching in the two groups was the same, but by twelve days of age the average weight of the broilers (160gms) was already twice that of the S line birds. The weight of the broilers increased rapidly in the first four weeks of life. The S line birds gained weight at a more constant rate throughout the growth period,the graph of body weight / age being almost linear. The broilers by six weeks of age were three times the weight of the S line fowls.

In the S line fowls the femur and tarsometatarsus from each limb were of equal length, but in the broilers the tarsometatarsus was frequently slightly shorter than the femur. Initially at hatching the long bones of the broilers and S line birds were of the same length. The rate of growth was more constant in S lines than in broilers. The long bones of the broiler fowl, from two until eight weeks of age, grew considerably faster than those in the S line fowls. Between three and four weeks of age the broiler long bones were growing 1.5 times the rate of those in the S line fowls. At ten weeks of age the broiler growth rate was slightly less than in S line birds and by twelve weeks it was only 50% of the rate in S line fowl. In 90% of the S line birds intertarsal angulation was valgus, and between 5 and 10 degrees. In broilers there was a greater range of valgus intertarsal angulation with

the majority of birds in the range of 5 to 15 degrees. External tibiotarsal torsion of between 0 and 15 degrees was present in 97% of S line fowl, but only 66% of the broilers were in this range. The arithmetic means and ranges of bone torsion from this study were reported separately (Duff and Thorp, 1985a). The above comparisons of some of the features in the broiler and S line fowls emphasize the difference between the phenotypic expression in the two genotypes.

Rapid skeletal growth has been associated with the development of orthopaedic disease in dogs (Hedhammer et al, 1974), feedlot cattle (Jensen et al, 1981) and pigs (Reiland, 1974; Goedegebaure et a 1980). A comparison between the ultrastructure of endochondral ossification in the broiler and leghorn failed to identify any differences between the two breeds (Howlett, 1979). In the present study the far greater rate of growth corresponded to marked increase in the frequency of physeal abnormalities. Reiland et al (1978) considered, in a comparison of broiler and leghorn fowl, that the hypertrophic zone of chondrocytes was less well organized in the broiler and there was a more irregular calcification of the matrix. The organization of the growth plate in the faster growing species of domestic animals (pig, turkey and broiler) is poorer than in their slower growing counterparts (Olsson, 1982).

The most frequent physeal abnormalities in the present study were in the proximal tibiotarsus, where areas of physeal thickening occurred due to a local interruption of endochondral ossification. Blounts disease in the proximal tibia of man is the

result of an interruption in the normal rate of endochondral ossification due to a dyschondroplastic type lesion. The disease results in the development of tibia vara (Pappas. 1967). There are two types, infantile and adolescent, both occurring at a time of rapid growth and frequently in children that are overweight (Langenskiold, 1980). Similarily in the broiler fowls, which were considerably heavier than the S line fowls, dyschondroplasia and the development of abnormal tibiotarsal torsion and angulation occurred at a time of rapid growth. In the rabbit, segmental destruction of the distal physis results in a rotational deformity of the femoral shaft (Axer et al, 1972). Segmental lesions of the proximal physis of the tibiotarsus in the broilers may have caused rotational deformities leading to internal torsion. In broilers of six to ten weeks of age the frequency of internal torsion in the tibiotarsus corresponded to a high incidence of physeal abnormalities.

The long bones of the broilers were growing rapidly at fourteen days of age. There were dyschondroplastic lesions in 50% of the proximal tibiotarsi of the broilers during this period of rapid long bone growth. In man the development of osteochondrosis is associated with periods of rapid growth, either the early growth spurt or adolescent growth spurt (Duthie and Houghton, 1981). Male dogs grow faster than female dogs and are affected twice as frequently with osteochondrosis (Olsson, 1977). In pigs, dyschondroplastic lesions develop more quickly in the rapidly growing animal (Jussila and Paatsama, 1972). There were elevated levels of growth hormone and somatomedin in pigs selected for

rapid weight gain (Lund-Larsen and Bakke, 1975). When growth hormone was administered to growing dogs, osteochondrosis was induced in the extremities of long bones, whose growth rate was also stimulated (Paatsama et al, 1971). It appears unlikely that these hormones have a direct effect on osteochondrosis, but lesions probably develop due to the increased growth rate of the long bones.

Dyschondroplasia, identified by areas of thickened physeal cartilage, occurred in many of the broiler fowls throughout the growth period. The extent of the dyschondroplastic lesions varied. Some lesions were small and limited to a small part of the growth plate. In others, physes were grossly thickened and lesions involved their entire area. The dyschondroplastic lesions appeared to grow and regress during development. In the pig a similar pattern of waxing and waning of dyschondroplastic lesions occurs (Hill et al, 1984).

The cartilaginous epiphyses were larger in the broiler fowls. The greater number of blind ending EVCs in the cartilaginous epiphyses of broiler fowls is probably a reflection of a greater nutritional requirement by the epiphyseal hyaline cartilage.

Broilers fed ad libitum were physically inactive, and similar lassitude occurs in pups fed ad libitum (Hedhammer et al, 1974). Exercise and joint movement permits the diffusion of nutrients through cartilage (Sokoloff, 1969). The articular cartilage in the growing broiler was thicker than in the S line fowls. The sub-articular networks of EVCs in the broiler fowl may be a response to inadequate diffusion through the thicker articular

cartilage, and a lack of assistance to that diffusion due to their physical inactivity.

In dogs at the site of tendon insertions, between the intratendinous vessels and the vessels of the cartilaginous epiphysis, there is an avascular zone (Bengin, 1980), In the present study there was a similar avascular zone, at the insertion of the iliotrochanteric muscle into the cartilaginous epiphysis on the lateral aspect of the femoral trochanter. The avascular zone was more extensive in the broiler fowls. A common cause of lameness in the broiler is avulsion of the iliotrochanteric muscle from its insertion. Duff (1985c) commented that the susceptibility of the femoral trochanter to muscle avulsion was by virtue of its predisposition to osteochondrosis and the pattern of muscle insertion. In the femoral trochanter retained epiphyseal hyaline cartilage was due to incomplete and irregular ossification, resulting in areas of chondrocyte death and cavity formation (Duff, 1985c). The greater extent of the avascular zone at the insertion of the iliotrochanteric muscle in the broiler, in conjunction with epiphyseal hyaline cartilage retention, must increase the likelihood of avascular necrosis being a major factor in the pathogenesis of avulsion at this site.

There was little difference in the developmental pattern of EVCs in the cartilaginous epiphyses of broiler and S line fowls. The EVCs were from the same sources in the broiler and supplied a similar area of the epiphysis as in the S line fowls. The main effect of breed difference was the larger cartilaginous epiphyses in the broiler fowls requiring the EVCs to be much more extensive.

In a similar fashion when there was an area inadequately supplied by EVCs then adjacent vessels compensated by extending into the poorly perfused cartilage. In the broiler the distance such compensating EVCs would have to traverse was greater.

The features of the PEVs in the broiler fowl were more diverse than in the S line fowl of the previous experiment. In the newly hatched chicks there was bifurcation and lateral branching of transphyseal PEVs. Once the growth plate was fully functional there was still a marked difference in the morphology of PEVs in comparison to the S line fowl. In the broiler fowls the PEVs in each physis showed a lot of variation in size. In the S line, transphyseal PEVs were only present until day 14, although they occurred frequently throughout the growth period in the broiler fowls. Broiler PEVs frequently showed transphyseal tails and lateral bulges of their walls. The PEVs of the S line fowl were all similar in shape, forming evenly spaced cylinders penetrating the physeal cartilage. PEVs in broiler physes showed variety of shapes. the occasional amalgamation, bifurcation and irregular spacing.

Broiler MVs also varied from those in the S line fowls. The MVs of the broiler were uneven in size and the depth to which they penetrated the physeal cartilage. The spacing between MVs varied as did the distance between the ends of the MVs and the ends of the PEVs. An increase in the distance from the PEVs to the MVs signified a build up of hypertrophied or prehypertrophied chondrocytes. Reiland et al (1978b) also considered that the pattern of vascular penetration of the physeal cartilage was more

regular in the leghorn type fowl than in the broiler fowl. In the present study, frequently there was disruption of MVs beneath regions of thickened physeal cartilage. The irregular and blunt ending MVs appeared unable to progress normally into the physeal cartilage. When such lesions were at the periphery of the physis/metaphysis then MVs as they attempted to advance around the retained physeal cartilage would cause a bulge in the external contour of the bone extremity. This was a cause of the misshapen bone extremities associated with dyschondroplastic lesions of physeal cartilage.

Hill (1984) reported foci of retained cartilage in the physis of 12 out of 21 pigs which were day old. These foci in the pig could be remnants of cartilaginous metaphyseal cores, similar to the cartilage cores in the newly hatched chick. If this is so then the cartilaginous foci in the neonatal pig cannot be considered as a form of dyschondroplasia.

One form of osteochondrosis in the dog involves thickening and degeneration of avascular cartilage adjacent to the joint cartilage, and is a failure of the EOC to ossify the expanding epiphysis (Olsson, 1976). The vast majority of cases are in large and giant breeds of dog, whose genetic capacity for rapid growth and being "pushed" nutritionally increases the risk of osteochondrosis (Olsson, 1976). The incidence and severity of osteochondrosis in the pig is greater in fast growing breeds (Grondalen and Vaughen, 1974). The thicker articular cartilage in some breeds of pigs predisposes them to osteochondrosis (Kincaid and Lidvall, 1983). In the present study the articular cartilage

in the broiler was thicker than in the S line fowls and the cartilaginous epiphyses were more extensive. At the end of the growth period the EVCs disappeared from the cartilaginous epiphysis which then ossified. In the broiler, because of the greater extent of the epiphyseal hyaline cartilage, the process of ossification takes longer. The occlusion of EVCs prior to epiphyseal ossification results in avascularity of the peripheral remnants of hyaline cartilage. The death of chondrocytes and matrix necrosis would result in osteochondrosis. In pigs the majority of osteochondrotic lesions occur towards the end of skeletal growth (Bhatnagar et al, 1981; Hill et al, 1984), at the time of sexual maturity (Grondalen, 1970). The retention of epiphyseal hyaline cartilage in the femoral head of broiler fowls, due to disturbed endochondral ossification, leads to "capital femoral osteochondrosis" (Duff, 1985b; Duff and Hocking, 1986). In the present study remnants of epiphyseal hyaline cartilage were present around the periphery of the epiphysis in broiler fowls of 20 weeks of age. The greater extent of such remnants in the broiler fowls would predispose them to osteochondrosis.

The vessels on the surface of the femoral head surrounding the fovea covered a more extensive area in broilers. This could signify a lesser degree of joint congruence in the broiler compared to the S line.

A frequent abnormality in the broilers was the occlusion of EVCs from the medial ICRVs of the femoral head. These lesions of the medial femoral head frequently occurred in broilers from 14 days of age. The avascular region in the craniomedial femoral

head was associated with either thickening of the physeal cartilage or areas of necrosis in the epiphyseal hyaline cartilage and physeal cartilage. In a study of avian dyschondroplasia in the femoral head Duff (1984a) identified eosinophilic scars and vascular canal occlusion at the site of increased growth plate thickness, commenting that patent vascular canals are required for chondrocyte hypertrophy. Thrombi causing occlusion of PEVs have been described at the site of osteochondrosis type physeal lesions in the pig (Kincaid and Lidvall, 1982). In capital femoral epiphyseal infarction of the fowl there was more extensive vascular occlusion than in dyschondroplasia (Duff, 1984b). Retention of physeal cartilage did not always occur in capital femoral epiphyseal infarction but necrosis did result. The medial periphery of the proximal femur was most vulnerable to EVC occlusion. The vulneribility of this site has been confirmed in the present study. In the present study the area of avascularity in the medial aspect of the femoral head was, in most cases, revascularizing with EVCs from the capital femoral ligament.

In the present study there was a change in MV alignment in some growth plates. This reflects a bending of cartilage columns between MVs. No changes in the alignment of MVs were noted in the S line fowls. When torsional forces were applied to the growing bones of the rabbit, by the application of a plaster cast, a deflection was produced in the cartilage columns (Arkin and Kanz, 1959). Similar bending of the cartilage columns was reported, by Duff (1986b), in sheep as a response to increased loading and shear stress. The greater weight of the broiler will cause the

physeal cartilage to be subjected to far greater torsional loading, considerably increasing the shear stress in the physis, and leading to bending of the cartilage columns and a change in MV angulation. In the present study the change in the alignment of the MVs suggests a change in the direction of bone growth, which occurs concurrently with a greater range of individual bone torsion and limb angulation in the broiler fowls.

In the present study clefts containing haemorrhage were reported in the physeal cartilage of a number of specimens. The clefts were in the prehypertrophied zone of chondrocytes and ran parallel to the direction of growth. In the rat, necrosis and fissures have been reported between the physis and epiphysis of the proximal tibia, and mechanical forces were considered essential to their aetiology (Yamasaki and Inui, 1985) The fissures prevent the passage of blood from the epiphysis to the physis leading to regressive changes in the physeal cartilage (Yamasaki and Inui, 1985). The present study suggests that stresses induced by the greater body weight of broiler fowls, may be a factor in the formation of physeal clefts.

Ganz et al (1981) demonstrated the susceptibility of areas in the femoral head of dogs to experimentally elevated intra-articular pressure. They suggested that elevated pressure could compromise the capsular vessels. In the present study there were a number of sites where it was common to see thickening of the physeal cartilage. These sites were in the lateral aspect of the proximal tibiotarsus, the medial and lateral aspects of the proximal tarsometatarsus, the medial and lateral aspects of the

distal femur and the medial aspect of the proximal femur. When lesions occurred at these sites the PEVs could be present and normally perfused, or were elongated or were occluded. At all these sites the PEVs were supplied by EVCs from ICRVs. which were intimately associated with the joint capsule. The result of a temporary embarassment of the blood supply to the EVCs there would be disruption of the differentiation of the chondrocytes in the physis and in the maintenance of cartilage matrix. The more rapid growth rate of the broiler fowl, by increasing the maintenance requirements of the physis, would increase susceptibility to such disruption. The broilers fed ad libitum spend much of their time seated on the floor. Abnormal limb posture for long periods of time may result in a restricted blood flow to the ICRVs.

EXPERIMENT 5:Broiler fowl fed at a restricted rate.

INTRODUCTION.

The previous experiments demonstrated that a similar pattern of cartilage canals was present in the cartilaginous epiphyses of S line and broiler fowls fed ad libitum. In the broiler fowls there was a high incidence of vascular abnormalities in physeal cartilage which were frequently associated with regions of physeal thickening and dyschondroplasia.

In domestic pigs the incidence of osteochondrosis, a form of dyschondroplasia, can be controlled by food restriction (Reiland, 1978b). When domestic pigs were mated to wild boars there was a reduction in growth rate, and virtually no osteochondrotic lesions (Reiland, 1974). Ljunggren and Reiland (1970) reported a positive correlation between the incidence of joint lesions and growth rate in the pig. The restriction of food intake in fast growing large breeds of dog reduced the incidence of lesions suggestive of developmental orthopaedic disease (Hedhammer et al, 1974). In feedlot cattle it was suggested that the incidence of osteochondrosis increases at higher growth rates (Jensen et al, 1981).

Edwards (1983) considered that the genotype of the fowl altered their susceptibility to dyschondroplasia; and a study of six different breeds of pig demonstrated however that the extent and severity of osteochondrosis was greatest in those with the

highest growth rates (Goedegebuure, 1980). Male breeding turkeys fed only 80% of the quantity of food ad libitum fed birds would eat showed a reduced incidence of dyschondroplasia (Steinke, 1971). Riddell (1975b) postulated that in the broiler, although growth rate was only a contributory factor in the development of dyschondroplasia, a slower growth rate reduced the incidence.

Restricted food availability is used commercially in the production of broiler breeding stock to reduce the incidence of skeletal disease causing lameness. Indeed the regime of feed restriction in the present study was identical to that used by D.B.Marshalls Ltd. (Newbridge, Midlothian, Scotland.) in the production of breeding fowl.

The purpose of this experiment was to examine the effect of feed restriction on growth and vascularity of the extremities of the pelvic appendicular skeleton in the broiler. Previous experiments in the present study have established that the most marked differences between physeal vasculature in broiler and S line fowls, and the highest incidence of physeal thickening was in birds of four, six and eight weeks of age. The present experiment was designed to prepare specimens from broilers fed a restricted ration enabling a direct comparison with the birds of the same agein the two earlier experiments. Hereafter in the present study broilers fed a restricted ration are termed restricted broilers.

MATERIAL AND METHODS.

Twenty four broiler chicks were reared from day old in floor pens. They were fed a commercial starter diet* (23% protien and 3000kcal ME) until four weeks of age. From two weeks of age the total daily quantity of food available to the group of birds was restricted. The feeding regime is outlined in Table C. The birds were all weighed weekly.

Eight birds (four male and four female) were killed at 28, 42, and 56 days. After killing, the birds were routinely weighed. After dissection the pelvic appendicular skeleton was radiographed in a Faxitron 804 using Kodak X-Omat RP film in a Kodak X-Omatic fine screen plate. Both AP and lateral views were taken. Estimates of torsion were recorded for the three long bones, by comparing the transverse axes of the proximal and distal articular surfaces (Duff and Thorp, 1985a and 1985b). The post mortem details, of age, sex weight, long bone torsion and long bone length, from each bird are recorded in Appendix 3.

Two specimens from each kill were processed in Polymaster resin. The Polymaster blocks containing the proximal femurs, proximal tibiotarsi and the proximal tarsometatarsi were subsequently cut into slabs and examined.

From the radiographs of all the restricted broilers at each kill the length of the long bones was measured, and as previously the rate of long bone growth calculated.

The number of bone extremities with physeal abnormalities were totalled and then calculated as a percentage of the total

* Further details of diets in appendix 5.

TABLE C.

Feed available per bird per day from two till eight weeks of age.

AGE(weeks)	DAILY QUANTITY OF FOOD AVAILABLE PER BIRD(gms)
2-3	45
3-4	50
4-5	50
5-6	50
6-7	54
7-8	54

number of bone extremities examined. The same calculations were performed on the bone extremities of the ad libitum fed S line and broiler fowls of the same ages. The mean torsions of the three long bones for each of the three groups of fowl were calculated (Table D).

Behavioural differences between restricted and ad libitum fed broilers were apparent. The restricted broiler fowls appeared more active than ad libitum fed broiler fowls. The ad libitum birds either ate or sat on the floor. The restricted birds were more alert and scratched around the floor of the pens.

The average weight of the restricted broilers until eight weeks of age was compared with the ad libitum fed broiler and S line fowls (Fig 113). The restricted broilers gained weight steadily through the experiment, at eight weeks of age the average weight was 881 grams. The rate of long bone growth in the three groups (ad libitum S lines, ad libitum broilers and restricted broilers) was compared at six weeks of age (Fig 114).

The percentage of abnormalities in the bone extremities of the three groups of birds was also presented in Fig 114. The vascular lesions were less extensive in the restricted broiler than in ad libitum fed broilers of the same age.

The estimated average torsions of the three long bones in the three groups of birds are presented in Table D. In the restricted broilers the average torsion of the tibiotarsus and tarsometatarsus varied little at each kill. The average femoral torsion was reduced by 4 degrees.

In fowls reared under feed restriction, the general pattern of vasculature supplying the epiphyseal hyaline cartilage was similar to that noted in the ad libitum fed broiler fowls. Differences were however more apparent in physeal vasculature in

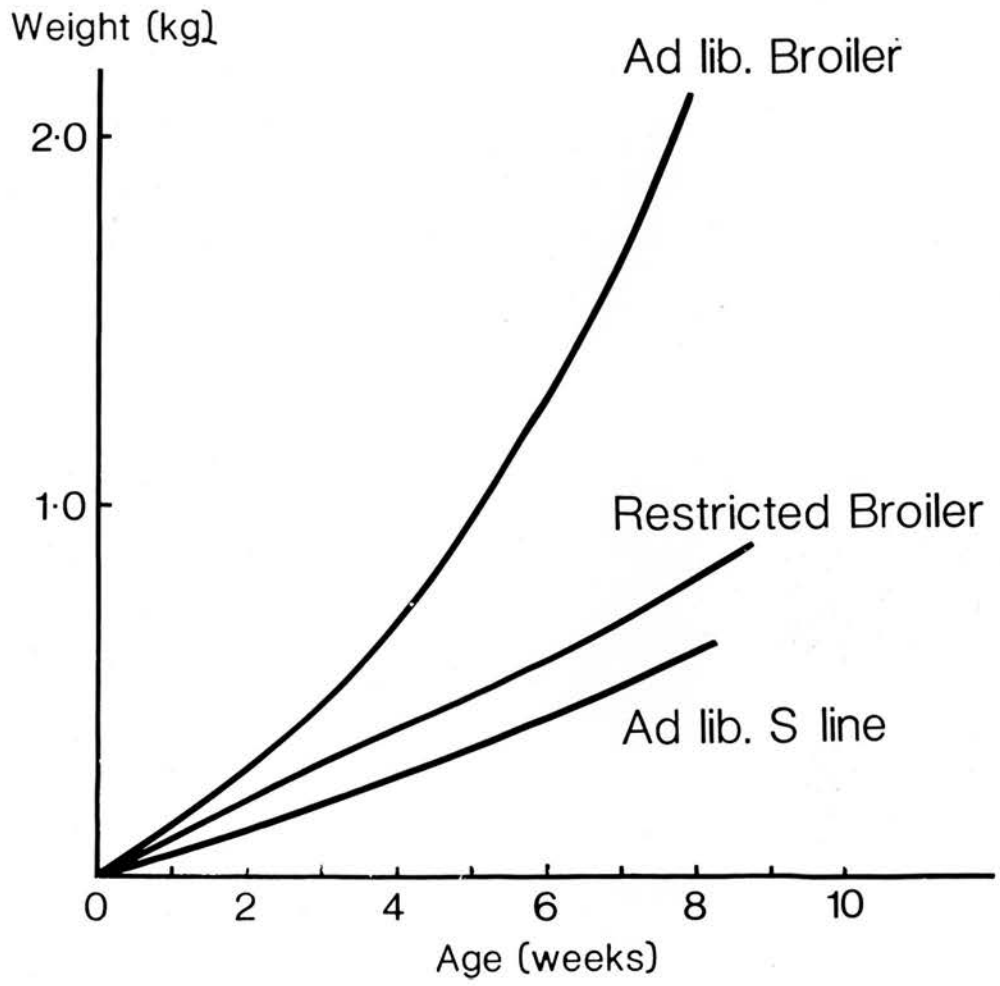


Fig 113. The average weight of ad lib fed broiler, ad lib fed S line and restricted broiler fowl from day old to 10 weeks of age.

TABLE D

The mean torsion of the long bones of ad libitum fed S line, restricted broiler and ad lib fed broiler fowls.

FEMUR (torsion degrees).

Age (weeks)	4	6	8
S line	13	14	12
Restricted broiler	15	11	11
Ad libitum broiler	8	8	10

TIBIOTARSUS

Age (weeks)	4	6	8
S line	10	9	8
Restricted broiler	6	6	6
Ad libitum broiler	4	3	0

TARSOMETATARSUS

Age (weeks)	4	6	8
S line	-11	-11	-10
Restricted broiler	-8	-8	-7
Ad libitum broiler	-10	-12	-12

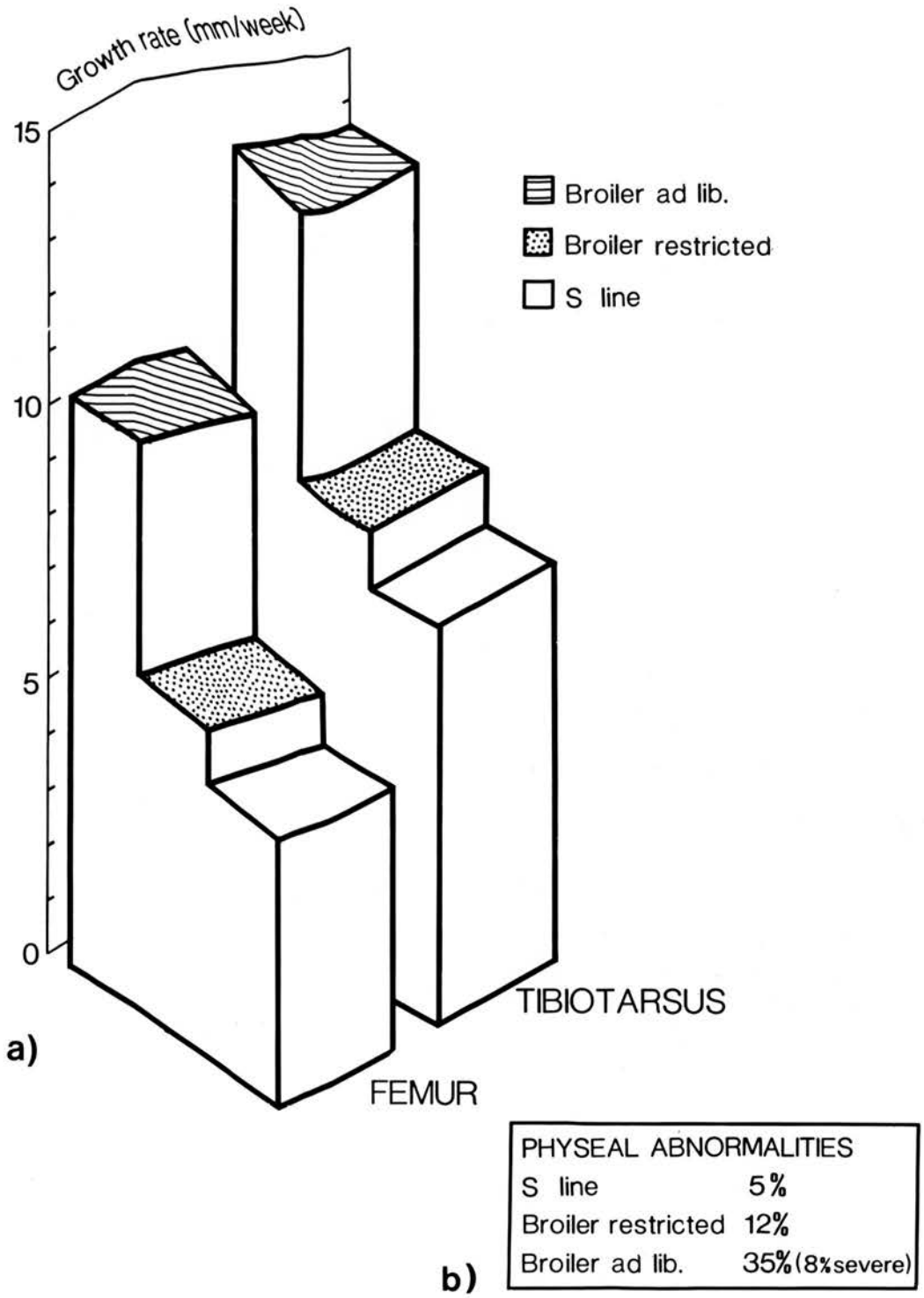


Fig 114. a) The growth rate at 6 weeks of age of ad lib fed broiler, ad lib fed S line and restricted broiler fowl.
b) The % of long bone extremities with vascular abnormalities in the same groups of fowl as a).

the two groups.

Vasculature and morphology of the restricted broiler fowls.

Four weeks.

In the medial aspect of one proximal tibiotarsus, and in the proximal tarsometatarsus from the same limb, there was thickening of the physeal cartilage. There was elongation of PEVs in association with both lesions and delayed MV invasion.

The vasculature of all the other bone extremities was considered to be normal, with even spacing in the arrays of PEVs and MVs.

Six weeks.

The PEVs and EVCs in all physes formed evenly spaced arrays. There was variation in PEV width and PEV tails were observed making contact with MVs in most of the bone extremities. There was less variation in the size of individual PEVs compared to the ad libitum fed broilers. Transphyseal connections between PEVs and MVs were a more frequent occurrence than in S line specimens of the same age. Occasional bifurcating PEVs occurred in the physeal cartilage of proximal femurs. In one of the femoral heads there were few medial EVCs and PEVs.

The thickness of the physeal cartilage was constant in most physes, but in one limb of one case there was mild peripheral thickening in the proximal tarsometatarsus. At the site of physeal thickening in this case the PEVs were normal in length and

there was a failure of MV penetration of the physeal cartilage.

One proximal tibiotarsus was slightly thickened across the entire width of the physis. The physeal thickening was greatest adjacent to the fibula. There were many elongated PEVs which made vascular contact with MVs. The MVs were uneven in size and some formed widened tracts which divided near the physeal cartilage.

Eight weeks.

In these birds there was little evidence of physeal thickening or vascular abnormalities. The arrays of PEVs and MVs were regular. In one proximal tarsometatarsus however there was a lesion which consisted of a peripheral delay in MV invasion and mild physeal thickening. In this instance PEVs were normal in length.

DISCUSSION

The restricted broilers were nearer to the average weight of the S line fowls than that of the ad libitum fed broiler fowls. The rate of long bone growth in the restricted broilers was also similar to the S line fowls. At six weeks of age the long bones of restricted broilers were growing 10% faster than those of S line fowls but at only two thirds of the growth rate of ad libitum broilers. The mean torsions in the restricted broiler fowls were very similar to those in the ad libitum fed S line fowls. In the ad libitum broilers the mean torsions of the femur and tibiotarsus were less than those of the other two groups. There were 13 tibiotarsi with internal torsion in the ad libitum broilers. Internal torsion did not occur in restricted broiler or S line fowls.

In the restricted broiler fowls the physeal vasculature was better organized than in ad libitum fed broiler fowls. The PEVs and MVs were in well ordered, evenly spaced arrays and the majority were of similar size. In the restricted broiler fowls slight physeal thickening was only occasionally noted. The more normal patterns of bone torsion and physeal vasculature in these restricted broilers occurred in conjunction with the absence of dyschondroplasia. The internal tibiotarsal torsion occurring in ad libitum broiler fowls may be caused in part by defective endochondral ossification recorded in the proximal bone extremity (Duff and Thorp 1985a). The lack of activity, in the lethargic ad libitum fed broilers, was probably a contributing factor in the

development of differing bone torsion, and is investigated in subsequent experiments in the present study.

Nakano et al (1984) restricted the diet of a group of pigs and compared them to ad libitum fed pigs of the same age. They found no effect of dietary restriction on the incidence of osteochondrosis. The first group of pigs gained weight at a rate of 0.72 grams per day. The second, ad libitum fed, group of pigs gained weight at a rate of 0.78 grams per day. This restriction only reduced weight gain by 8%. In the present study the restricted broiler fowls were only 50% of the weight of the ad libitum broiler fowls, and there was a marked reduction in the incidence of physeal abnormalities.

The difference in the physeal vasculature between restricted and ad libitum broiler fowls suggests that genotype is not the primary cause of increased susceptibility to dyschondroplasia. The expression of genotype by increased growth rate however can manifest as an increase in the number and severity of dyschondroplastic lesions in the fowl.

The number of birds examined in the present study was small, but the results show a remarkable transformation in the broiler fowls. The birds fed at a restricted rate assumed many of the characteristics of physeal vasculature of the S line fowls, which may indicate a reduced susceptibility to osteochondrosis (dyschondroplasia).

EXPERIMENT 6:The effect of exercise on the vasculature of the long bone extremities.

INTRODUCTION.

Experimental evidence suggests that functional activity is of value in the maintenance of cartilage and bone. Bone rarefaction, induced by circulatory stasis when the limbs of rabbits were placed in plaster casts, was relieved by Faradic stimulation of the associated muscles (Geiser and Trueta, 1958). In cartilage the diffusion of nutrients is promoted by exercise or joint movement (Sokoloff, 1969). The majority of research into the effect of functional activity has centred around articular cartilage. Adult articular cartilage reacts to load (Saaf, 1941), and the chondrocytes hypertrophy in response to non strenuous activity (Paukkonen, 1985). In the young pig, exercised daily from three weeks until five months of age, there was a stimulatory effect on the process of growth and regeneration of articular cartilage (Saaf, 1950).

When the limbs of rabbits were immobilised in plaster casts there was a reduction in the effective capillary circulation (Hulth and Windborn, 1963). The effect was temporary, lasting for 24 to 48 hours, and manifested by fewer erythrocytes in the capillaries and reduced matrix uptake of a metabolic marker (S35). In the experimentally immobilised canine limb, proteoglycans were not maintained in the articular cartilage (Palmoski et al, 1979).

Further work suggested that loading and joint motion were required for the sustenance of the proteoglycans of articular cartilage (Palmoski et al, 1980).

The comparison of different methods of husbandry also enables an assessment of the effects of activity on growing bone. Individual confinement of growing pigs produced a greater incidence of lamness (Elliot and Doigne, 1973). A similar investigation found that the extent of the cartilage lesions was related to the duration of confinement and not to the terminal weight of the pigs (Fredeen and Sather, 1978). The results of a clinical study on 257 immature pigs it was suggested that confinement housing was a major contributory factor in the occurrence of "leg weakness" (Sather and Fredeen, 1982) When young boars were exercised in a treadmill the incidence of clinical signs including aberrant limb angulations, was reduced (Perrin and Bowland, 1977). There was a greater incidence of cartilage lesions in growing bulls kept under conditions of confinement (Siebel et al, 1973).

Rodenhoff and Dammrich (1971) in a comparison of free range and housed cockerels from 14 days of age, commented that a "better skeleton" resulted in free range birds as a result of increased exercise. In a later study, cortical bone from the femur was compared in birds reared under different conditions of husbandry (Rodenhoff and Dammrich. 1973). The "best bone" was produced in birds kept under free range conditions. Articular cartilage lesions in the distal tibiotarsus of turkeys have been described as following inactivity due to severe footpad dermatitis (Julian

and Bhatnager, 1983).

Hester et al (1983) made subjective evaluations on activity in fowls kept under different lighting regimes. Lameness in the flocks was assessed by observation and post mortem evaluation of casualty birds. This investigation suggested that stimulation of activity by intermittent light patterns reduced the incidence of leg abnormalities. In a later study (Hester et al, 1986) a reduced incidence of limb abnormalities was again reported in association with specific lighting patterns. Bird activity was measured with radar techniques in a study of the effect of different lighting regimes on the incidence of lameness in growing broiler fowls (Simons and Haye, 1985). Intermittent lighting, which increased bird activity throughout each 24 hour period, reduced the incidence of "twisted leg". Wilson et al (1984) similarly found significantly fewer and less severe leg abnormalities in broilers grown under intermittent lighting patterns.

Pigs do not exercise volantarily and if fed ad libitum spend 70 - 80% of their time resting. A feed restriction of 15% causes the pigs to be active 50% of the time (Ewbank, 1974). The fowl has been monitored in trials to establish the percentage of energy intake that was utilised in physical activity (Wenke and Es, 1977). The ad libitum fed fowl only utilised 7% of energy intake in physical activity. Similar fowls when restricted in feed availability by 25% utilize 15% of energy intake in physical exercise (Wenke and Es, 1977). It can be concluded that restricted birds are likely to be more active than ad libitum fed

fowls. The increased physical activity in the restricted broilers of the previous experiment may have contributed to some of the differences between them and ad libitum fed broilers.

The purpose of the present experiment was to assess the effect of regular exercise on the vascular morphology of long bone extremities in ad libitum fed broiler fowls.

MATERIAL AND METHODS.

A carousel was used to exercise the birds in this experiment. The carousel of wire cages was rotated by an electric motor (Fig 116 and 117). The platform under the cages remained stationary. A rheostat was used to vary the current to the electric motor and hence alter the speed. Initially, when the birds were first introduced to the carousel, a slow speed of rotation was used. As the birds adapted to walking round the carousel's platform the pace was gradually increased.

Forty eight male broiler fowls were reared from day old in deep litter floor pens and fed ad libitum. The broiler fowls were grand parent broiler breeder stock supplied by D.B.Marshalls. The diets used were similar to those in the previous experiment, as was the lighting regime. The birds were divided into two groups of twenty four, group A and group B. The total daily food consumption by each group was measured. Group A were exercised daily on the carousel for four periods of fifteen minutes. The non-exercised birds, group B, were caught and confined in a dark box for the duration of each exercise period.

Birds were exercised from eight days of age. After five days of exercise four birds from each group were killed. After twelve days exercise a further six exercised and four controls were killed The remaining birds were killed 25 days after the start of exercise, when the birds were four and a half weeks of age. The birds were all weighed weekly and after killing. The average weight was calculated for each group at two, three and four weeks

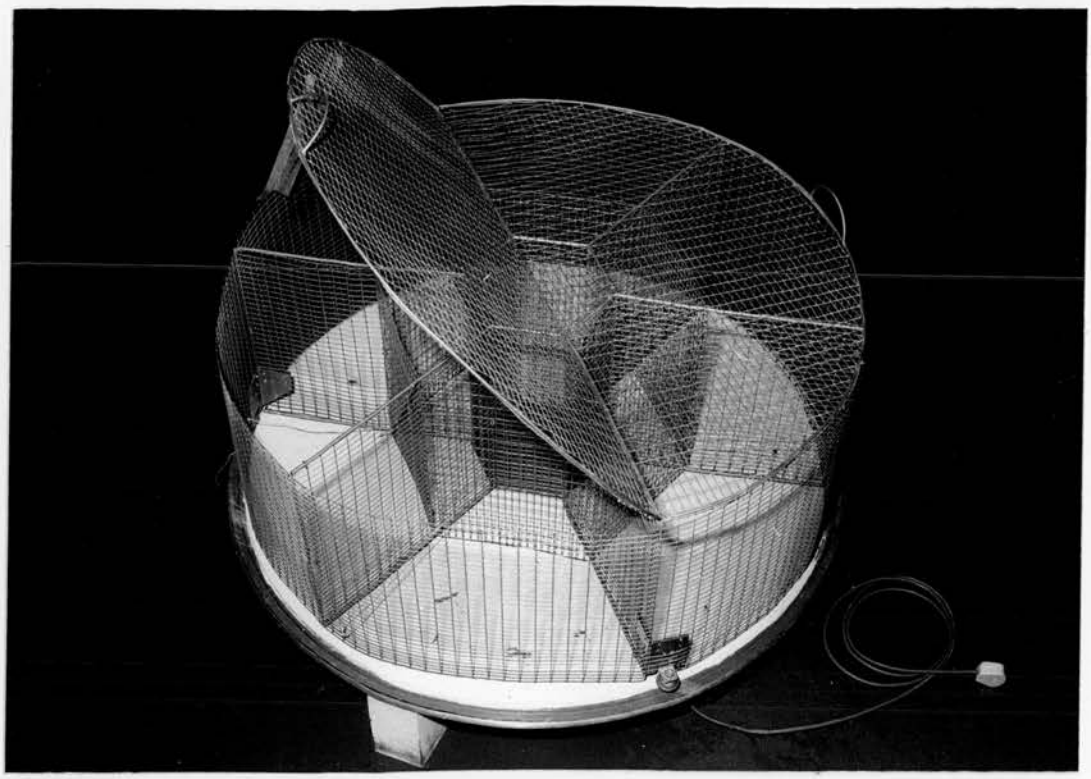


Fig 115. The exercise carousel.The cages rotate driven by an electric motor below the platform.

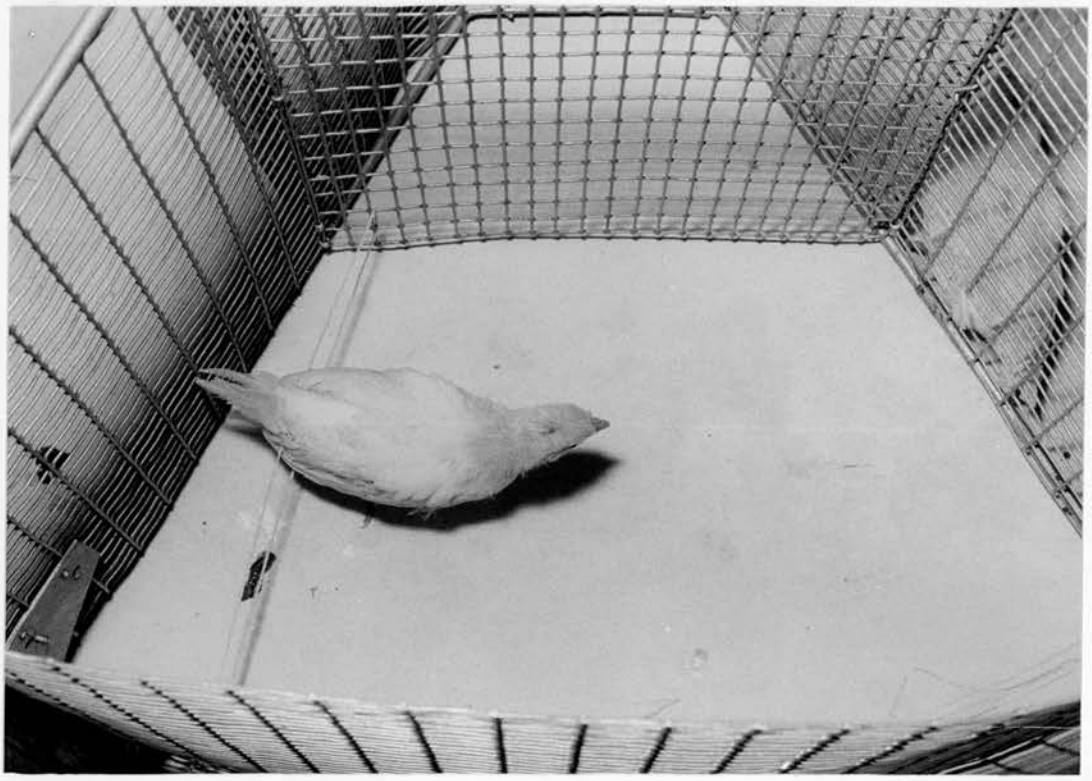


Fig 116. An 18 day old S line is being exercised in the carousel as the cages rotate.

of age.

The proximal bone extremities from the limbs of twenty three exercised and twelve control birds were embedded in Polymaster and then cut into slabs.

At first the carousel was rotated at one revolution each 80 seconds but, as the birds became familiar with the moving cages, speed was increased to one revolution every 30 seconds. This is equivalent to a pace of six metres per minute. Some of the birds, towards the end of the fifteen minute exercise period, would sit down on the floor of the carousel. When they were nudged by the back wall of a cage the sitting bird would stand and run forward to then sit down again. During the periods of exercise the birds did not appear fatigued or distressed.

On the second day of exercise one bird persisted in sitting down and was then being pushed by the moving carousel. This bird was returned to the floor pen. The bird was still unco-operative on the third day of exercise and was removed from the experiment.

The average weight of the birds in each group was calculated (Table E). From these figures the average weekly rate of weight gain could be calculated (Table F).

The average weekly weight gains and the total weekly food consumption in the two groups were used to calculate the weekly food conversion ratio (FCR) (Table G).

The average weight gain in the exercised broilers was approximately 5% less than that of the non-exercised birds. There was no significant difference in the FCRs between the two groups.

TABLE E

The average weight of birds in each group.

	WEIGHTS(gms)		
	2weeks	3weeks	4weeks
Exercised	205	399	636
Control	207	417	669

TABLE F

Average gain in weight per week per bird (grams).

	2 - 3 weeks	3 - 4 weeks
Exercised	194	247
Controls	210	254

TABLE G

Food conversion ratios.

	2 - 3 weeks	3 - 4 weeks
Exercised	1.85	1.86
Controls	1.96	1.83

13 day old

There were abnormalities in five bone extremities.

In two proximal tibiotarsi and in one femoral head there was variation in the size and spacing of MVs. In these cases the depth of MV penetration of physeal cartilage was irregular. There were localized areas of physeal thickening due to delayed MV penetration in one proximal tibiotarsus and one tarsometatarsus.

20 day old.

There were abnormalities in five bone extremities.

The MVs in the lateral condyle of two proximal tibiotarsi were thickened, blunt ending and formed irregular arrays. There was an area of dyschondroplasia in the medial condyle of one proximal tibiotarsus and MVs were branching around the retained physeal cartilage. In two femoral heads the MVs were uneven in their depth of penetration of physeal cartilage.

33 day old

There were abnormalities in eight bone extremities.

In one proximal tibiotarsus the MVs were uneven in size and in their depth of physeal penetration. Six proximal tibiotarsi contained localized areas of dyschondroplasia in the physeal cartilage of the lateral condyle adjacent to the fibula. The MVs below the thickened dyschondroplastic physes were thickened and

blunt ending. There was a cleft containing haemorrhage in the physis of one femoral head. The cleft was perpendicular to the direction of growth and extended into the physis of the femoral neck.

THE BONE EXTREMITIES OF NON-EXERCISED BROILER FOWLS.

13 day old.

No lesions occurred in this group.

20 day old.

There were abnormalities in five bone extremities.

In one proximal tibiotarsus and in one proximal femur there was disorganisation of the MVs. They were unevenly spaced and some were of increased diameter. In three proximal tibiotarsi there was physeal thickening due to delayed MV invasion in the lateral condyle.

33 day old.

There were abnormalities in eighteen bone extremities.

In eight proximal tibiotarsi there were dyschondroplastic lesions in the lateral condyle adjacent to the fibula. The lesions were all similar with thickening and delayed MV erosion of the physeal cartilage. The MVs were blunt ending and unevenly spaced. PEVs were present in the physis of all the lesions, and although some were of normal length, others were elongated and extended into the thickened physeal cartilage.

In a further three specimens there was generalised thickening of the physeal cartilage in the proximal tibiotarsus. In two of these cases abnormal thickening was more severe in the lateral physis. In a further two proximal tibiotarsi there where areas were the MVs were uneven in size and variable in depth of penetration into the physeal cartilage.

The cartilaginous epiphyses in two craniomedial femoral heads were avascular and PEVs were absent from the underlying physes. In one of these the cartilaginous epipysis was being revascularised by EVCs which formed a "starburst" of vessels descending from the capital femoral ligament into the avascular cartilage.

The physis in one femoral trochanter was thickened and the MVs were of increased diameter and unevenly spaced. In a similar trochanteric lesion in another bird there was an absence of PEVs.

There was a cleft containing haemorrhage, perpendicular to the direction of growth, in the physis of one proximal tarsometatarsus. The peripheral physeal cartilage of another tarsometatarsus contained areas of thickening. Some of the PEVs in the thickened physis were elongated.

In the controls there were 24 sites in the bone extremities where vascular abnormalities occurred. In the exercised birds there were only 18 such areas. Abnormalities were recorded in 66% of bone extremities in control birds, but in only 25% of exercised birds (Table H).

In the examination of the bone extremities the impression gained was that there was a greater regularity and uniformity to

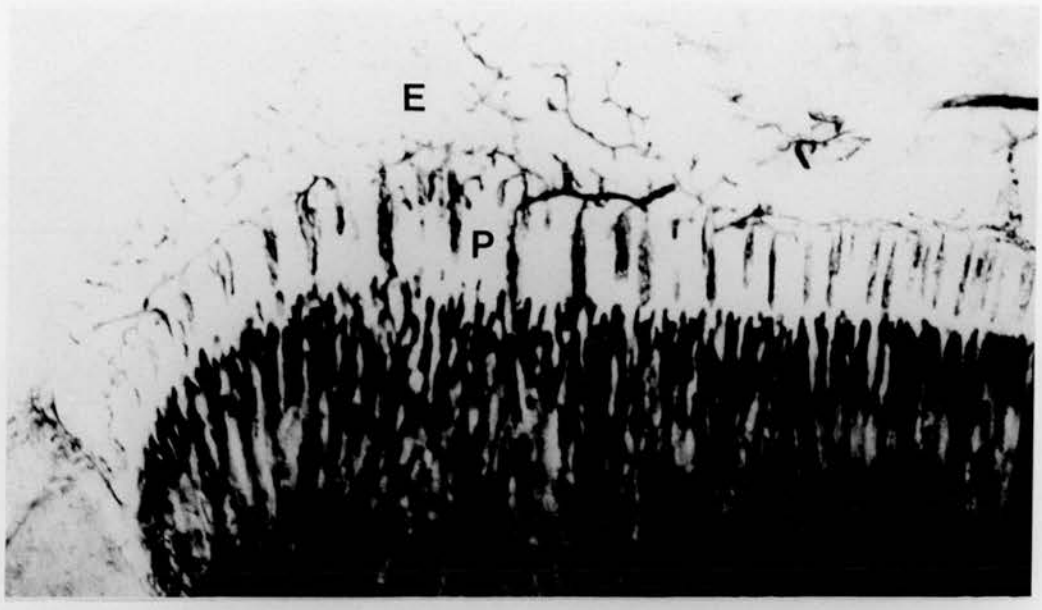


Fig 117. The medial condyle of the proximal tibiotarsus from a non-exercised broiler of 32 days of age. The MVs and PEVs are in uneven, irregular arrays. 1mm slab x16.

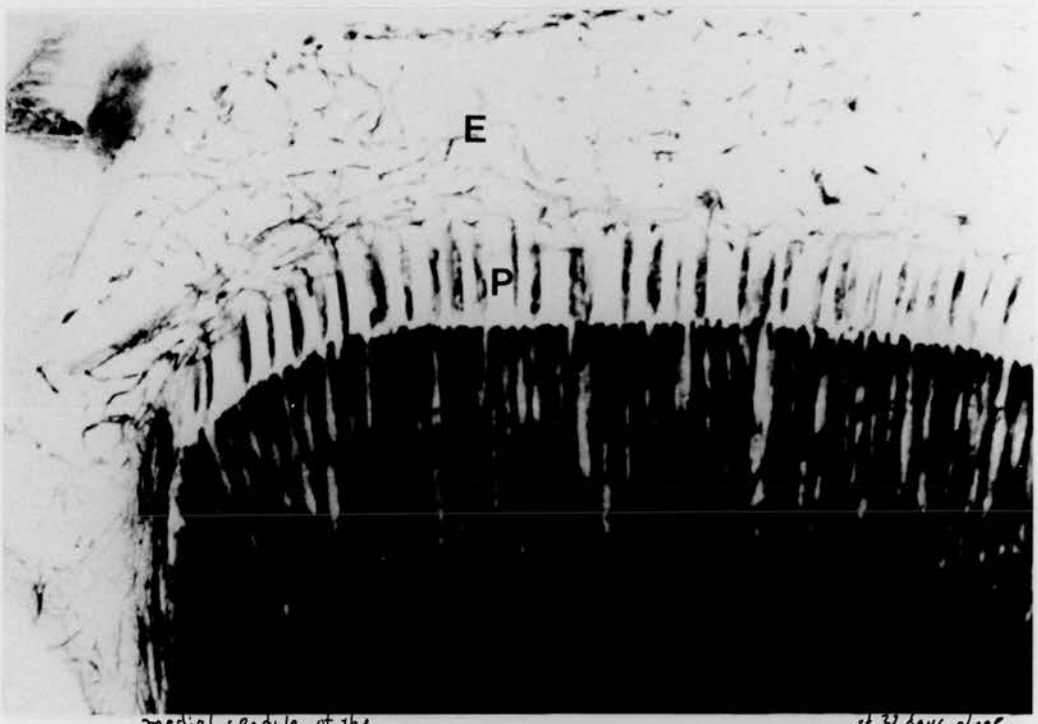


Fig 118. The proximal tibiotarsus from an exercised broiler. The 1mm slab is cut from the same site as in fig 117. The PEVs and MVs are in more regular arrays and more consistant in size than those of the non-exercised speciman in fig 117. 1mm slab x16.

TABLE H

Physeal and vascular abnormalities in the bone extremities

	No. of bone Extremities	No. of abnormalities	Percentage of abnormalities
CONTROL	36	24	66%
EXERCISED	72	18	25%

the arrays of PEVs and MVs in the exercised specimens (Fig 118) compared to the non exercised controls (Fig 119). The severity and extent of the abnormalities tended to be greater in the control specimens than in the exercised birds.

The carousel provided an effective means of quantifiably exercising birds on a regular basis. In this study the non-exercised birds were disturbed each time the experimental group were exercised. The effect of this was an increase in the activity of the normally lethargic broiler fowls. The controls (non-exercised) broiler fowls were probably more active than birds reared normally. They had however one hour less of activity per day than the exercised birds.

There was little difference in the weight gain of the two groups. In the last week of the experiment the non exercised birds only gained 7 grams more in weight than those which were exercised.

Exercise reduced the number of abnormalities occurring in the physeal vasculature. The effect of exercise on the bone extremities was similar to those of feed restriction though not as marked. The physeal vasculature tended to resemble that of the S line fowls recorded in experiment 3.

There were two principal factors which probably contributed to the improved physeal vascularity. The negative effects of inactivity were reduced and the positive effects of activity were promoted. Long periods of floor sitting could compromise the blood supply to the EVCs. In the immature dog the position of the hip can reduce blood flow to areas of the femoral head (Law et al, 1982). A quantitative investigation of blood flow was made in the femoral head of 11 pups, using the hydrogen washout method, When

the hip was held in relaxed abduction there was a significant reduction in femoral head circulation (Schoencker et al, 1978). It can be postulated therefore that prolonged periods of sitting in the broiler may result in temporary occlusion of EVCs.

Normal skeletal function (ie. activity) causes strains which alter blood flow and muscle pressure on periosteal surfaces deforms the bone tissue (Lanyon and Bourn, 1979). During locomotion each bone is subjected to one or more definitive loading phases. During loading phases each of the various parts of the bone are deformed and released in one particular direction (Lanyon and Baggott, 1977). In the fowl it can be suggested that the deformation and release of bone will cause a pumping action through the vascular canals and vessels in the bone extremities. There will be an improvement in tissue perfusion.

The more rapid growth of broiler fowl, compared to the S line fowl, would increase the vascular requirement for the maintenance of growth of cartilage. The resistance of cartilage to vascular insufficiency would be less in the faster growing fowls. The sedentary behaviour of the ad libitum fed broiler fowls appears to be detrimental to efficient vascular perfusion of the bone extremities.

EXPERIMENT 7:The effect of exercise and feed restriction on the development of bone torsion.

INTRODUCTION.

The previous experiment demonstrated that exercise had a modifying effect on the development of aberrant physeal vasculature and thickened physeal cartilage. A difference in the development of torsion in the three long bones of the appendicular skeleton in ad libitum fed broiler fowls and S line fowls was reported in the previous experiments. The occurrence of physeal abnormalities was associated with the development of a greater range of estimated bone torsion in the ad libitum fed broiler fowls when compared to S line birds. It has been suggested (Duff and Thorp, 1985b) that disturbed endochondral bone growth may initiate or exacerbate torsional loading in some bone extremities.

The purpose of this experiment was to establish the combined effects of food restriction and exercise on the development of bone torsion in broiler fowls.

MATERIAL AND METHODS.

Forty four male chicks were reared from day old in deep litter floor pens. The chicks were of similar genotype (D.B.Marshalls M4 strain). The birds were fed ad libitum until two weeks of age. They were then divided into two groups (A and B) each of twenty two birds, which were placed in adjacent floor pens. Group A were fed ad libitum, group B were fed at a restriction of 45 grams per bird per day.

The two groups were further divided at three weeks of age. Twelve birds each from groups A and B became groups C and D. Groups A and C continued to be fed ad libitum, whilst groups B and D were fed at a restriction of 50 grams per bird per day. The four groups were kept under identical environmental and husbandry conditions. Two of the groups (C and D) were exercised daily for four periods of fifteen minutes on the exercise carousel, in an identical manner to the previous experiment. All the birds were killed at five weeks of age, by which time groups C and D had received 14 days of exercise.

The birds were all weighed and the torsion of the three long bones estimated by comparing the transverse axes of the proximal and distal extremities. The average weight and average torsions were calculated for each of the four groups.

An index of the mean sum of the difference in torsion of the long bones of the right and left limbs was calculated for the four groups. The difference between the torsions of each pair of the three long bones in an individual bird was added. This

figure gave an indication of asymmetry in the limbs of an individual bird. The mean of the sum then provided an average of limb asymmetry for the group of birds. The resulting figure could in theory be compared with other groups to assess which group showed the greatest amount of torsional asymmetry of the pelvic appendicular skeleton.

There were no problems familiarising the chicks with the exercise carousel. The chicks fed a restricted quantity of feed appeared more active than the ad libitum fed birds.

The average weight of the birds in each group was calculated (Table I). The two ad libitum fed groups had an identical average weight. The restricted birds which were exercised weighed less than the restricted non-exercised birds.

The average torsion of each of the long bones was calculated in the four groups (Table J). There was little difference between the average torsions of the ad libitum and restricted broilers in the two non-exercised groups, the same was true of the two groups of exercised birds. When the exercised and non-exercised birds were compared it was found that the average external femoral and tibiotarsal torsion was less in the exercised birds.

The torsions all demonstrated the same pattern of variation between the two limbs of each individual. The average left femoral, left tibiotarsal and right tarsometatarsal torsions were greater than the average torsions measured in the opposite limb in all four groups.

The mean of the sum of the difference between the torsion in each pair of bones was greatest in the non-exercised ad libitum fed birds and least in the exercised birds which were fed a restricted quantity of food (Table K).

TABLE I.

The average weight of the birds in each group.

GROUP	DESCRIPTION	WEIGHT(GRAMS)
A	ad libitum, non-exercised	1060
B	restricted, non-exercised	690
C	ad libitum, exercised	1060
D	restricted, exercised	620

TABLE J

Index of the average difference between right and left long bone torsions in the four groups.

GROUP	TORSION(degrees)
Non exercised, ad libitum	20
Non exercised, restricted	18
Exercised, ad libitum	14
Exercised, restricted	13

TABLE K

The average torsion in each group.

	NON EXERCISED		EXERCISED	
	Ad lib	Restricted	Ad lib	Restricted
FEMUR				
left	20	20	16	18
right	14	14	13	14
TIBIOTARSUS				
left	7	4	3	3
right	-3	-1	-1	0
TARSOMETATARSUS				
left	9	8	9	9
right	15	14	13	13

In all four groups the average torsion was greatest in the long bones of the left limb. This confirms earlier reports by Duff and Thorp (1985a and 1985b), which suggested that limb dominance in domestic poultry may induce different torsional patterns in each limb. There were no apparent differences in the average torsional values of the different groups of fowls in this experiment.

The difference in the mean of the sum of individual torsional estimates was reduced sequentially by fed restriction and exercise. In earlier experiments feed restriction or exercise were both shown to improve the physeal vascularity and reduce the incidence of physeal abnormalities, the principal benefit being a reduction in the occurrence of disrupted endochondral bone growth. Disrupted endochondral bone growth in the previous experiments of the present study was considered to be a factor in the development of aberrant bone angulations and torsions. Asymmetry between the limbs could be expected as a result of aberrant torsion in individual long bones. The results of the present study would suggest that the improved physeal vasculature caused a reduction in the incidence of disrupted endochondral ossification, which in turn reduced the occurrence of aberrant torsion in individual long bones.

EXPERIMENT 8: The rate of growth at normal and dyschondroplastic bone extremities.

INTRODUCTION.

Stephen Hales (1727) first demonstrated that long bones grow by the addition of new tissues to the ends of the shafts, and not by general interstitial expansion. He drilled two holes in the tibiotarsal diaphysis of a chick and the distance between the two holes was the same two months later. Furthermore he noted that the length of the tibiotarsus had increased by one inch and that most of the growth had occurred at the proximal end.

The advent of radiology resulted in a modification of Hales' method. The position of a metallic marker, positioned in the diaphysis, could be measured on a radiograph. Bisgard and Bisgard (1935) drilled holes in the shafts of long bones in goats and then placed steel shot in the holes. Radiographs were taken and they measured the position of the marker relative to the proximal and distal bone extremities. The radiographs were repeated at the end of the experimental period, and the total contribution to growth by each extremity was revealed. A similar method, utilised by Sissons (1953) in a study of skeletal growth in rats and rabbits, placed metallic markers in femoral diaphyses. Radiographs were taken throughout the experimental period, enabling the accurate measurement of growth rate in the distal femur. Reidy et al (1947) in a study of radial and tibial growth in the dog also used

metallic markers.

A number of non-invasive, non-surgical methods have been developed to investigate the proportional growth rate of long bone extremities.

The feeding of madder, a red intravital bone stain, was the method used by Payton (1932) to measure the growth increments of each bone extremity in the limb bones of pigs. The madder was fed for a fixed period of time, then stopped. After a period of growth the animals under investigation were killed and the bones were sectioned longitudinally. The distance between the madder stained bone and the growth plate was measured, giving an estimate of linear growth in that extremity. Other intravital bone stains have been used in studies on bone growth. In particular fluorochromes have been employed. They are compounds that become incorporated in bone during its deposition and can subsequently be visualised under ultraviolet light. Intraperitoneal injection of a fluorochrome (tetracycline) was used in a study of bone growth in the rat (Hansson et al, 1972).

In the fowl tetracycline was used to estimate the growth rate of bone extremities at three and five weeks of age (Riddell, 1975). It was noted that the florescent lines were less well defined in the fowl than in comparable mammalian studies. Church and Johnson (1954), as part of their study of growth in the long bones of the fowl, gave an intraperitoneal injection of a flurochrome (alizarin). To estimate growth rate they measured the distance from the alizarin line to the growth plate. When Church and Johnson (1954) investigated growth in the humerus of the fowl

a pin was inserted in the mid-diaphysis. There is no reference in their report as to why a pin was used in the humerus, and they made no comparison between the technique of intravital staining and placement of metallic markers.

A novel technique was developed by Digby (1916) to estimate the proximal and distal growth of long bones. His assumption was that the nutrient canal points to the site of initial ossification. The bone was sectioned longitudinally and an imaginary line drawn along the nutrient canal to transect the centre of the medullary cavity. The distance from this point to each bone extremity was then measured. The results appeared to be a reasonable approximation to the proportional growth of the proximal and distal bone extremities. Church and Johnson (1954) confirmed their intravital staining findings using a modification of Digby's technique. They measured the distance from the nutrient foramen to each bone extremity but failed to comment on their results.

Harris (1926) examined serial radiographs of childrens feet over a number of years. He noted that there were transverse striations in the radiographs of individual long bones which remained as permanent features. He considered that the transverse striations were a reliable feature and could be used in the estimation of bone growth. Harris (1926) measured the distance from the striations to the bone extremity. He then repeated the measurement after a period of growth. An estimation of growth was made at that extremity by comparing the distances recorded. This method was refined by Roche (1963). Similarly, he used the

permanent radiological features of growth arrest lines (Harris lines) and fixed trabecular patterns in an investigation of metacarpal and metatarsal growth rates in man.

The incidence of vascular abnormalities varies, as does the development of pathological lesions in different bone extremities. Some disorders of the developing skeleton have been linked to rapid growth (Teare et al,1980). It is necessary to know the relative growth rate of each physis before an abnormality can be linked to more rapid growth.

Dyschondroplasia of the distal ulna in giant breeds of dogs (Riser and Shirer, 1965; Johnson, 1981) and in wolves (Ossent et al, 1984) has been associated with retarded endochondral ossification, leading to limb deformity. Olsson (1975) considered that physeal dyschondroplasia was responsible for retarded bone growth. Unequal growth in the proximal tibia of man, due to osteochondrosis, leads to a varus deformity (Pappas, 1967).

It has already been suggested in the present study that defective endochondral ossification may lead to the development of abnormal bone torsion. There may be a localized delay in endochondral ossification at the sites of abnormal physeal thickening (dyschondroplasia). Segmental damage in the physis of the distal femur in rabbits causes torsional deformities to develop (Axer et al, 1972).

The first part of the present experiment was designed to establish rate of growth in bone extremities of the normal S line fowl. This was followed by an assessment of the effect of physeal dyschondroplasia on longitudinal bone growth.

a) Normal S line fowls.

Ten S line chicks were reared from day old on deep litter. At thirteen days of age the birds were anaesthetised using halothane. Metallic implants were then inserted unilaterally into the mid-diaphysis of each long bone. A twenty three gauge needle (B-D, Microlance) was passed transcutaneously into the diaphyseal cortex. The protruding needle and hub was trimmed with a pair of pin cutters. A pair of forceps were then used to push the pin deeper so that the end of the implant lay subcutaneously. Whilst still anaesthetised the operated limb was radiographed. The bird was placed in lateral recumbancy with the operated limb next to the x-ray cassette. Positioning was such that the limbs did not overlie (Fig 119).

Two weeks post-operatively all the birds were killed. The hind limbs were then dissected and the bones were radiographed (Fig 120). The position of the metallic markers relative to the proximal and distal articular surfaces of the three long bones was measured in the post-operative and post mortem radiographs from each bird. The distances measured at thirteen days were subtracted from those at twenty seven days, the difference being the growth of each extremity for the thirteen day period. The rates of growth at each bone extremity were used to calculate the proportion of growth by each physis.

Fig 119. The radiograph of an S line taken post operatively. A metallic implant has been placed as a marker in the mid diaphysis of the 3 long bones of the pelvic appendicular skeleton.

Fig 120. The disected long bones from the same bird as in fig 119. This radiograph was taken when the bird was killed 2 weeks post operatively.

b) Broiler fowls.

Experiment "a" above was repeated with 17 male broiler chicks, reared from day old. The broilers however were grown in brooders throughout the experimental period. At 14 days of age the broilers were anaesthetised, and as previously metallic implants were inserted into the mid-diaphyses of the three long bones. Eight days post-operatively, when the birds were 22 days of age, all the broilers were killed. As previously the rate of growth at each bone extremity was calculated from the radiographs taken at 14 and 22 days of age. In radiographs, dyschondroplastic lesions were evident in some of the of individual long bone metaphyses.

RESULTS.

a) Normal S line fowls.

One bird died under anaesthesia during this experiment. The pins were not located properly in the femoral diaphysis of bird number 70 or the tibiotarsus of bird number 73. The metallic implants were still firmly embedded in the diaphyses of the long bones of all the other birds killed. The growth at each individual bone extremity for all the experimental birds is detailed in Table L. There was little individual variation between different birds. The growth occurring at an individual extremity was within 1mm of the average growth. The greatest range of growth occurred in the bone extremities with the most growth. The fastest growth rates occurred in the proximal tibiotarsus and proximal tarsometatarsus, which both grew an average of 10.4mm during the experimental period. The least growth (3.4mm) was in the distal tarsometatarsus. The distal tibiotarsus grew slightly faster than either the proximal or distal femur. The proximal and distal extremities of the femur grew at the same rate.

The average percentage of growth occurring at the proximal and distal end of the three long bones was calculated (Table M).

TABLE L

Growth(mm) at the extremities of the long bones of S line fowl between thirteen and twenty seven days.

	FEMUR		TIBIOTARSUS		TARSOMETATARSUS	
BIRD	prox	dist	prox	dist	prox	dist
68	7	6	10	7	11	3
70	-	-	11	7	10	4
72	7	6	11	7	12	4
73	5	5	-	-	9	3
74	6	6	10	7	11	3
75	7	7	9.5	6.5	10	3.5
76	6	6	11	7	11	3
78	5	6	10	6	9	4
79	6	6	11	7	11	3

KEY

prox = proximal

dis = distal

TABLE M

The average percentage of growth at the proximal and distal bone extremity in the three long bones of S line fowl.

	Femur	Tibiotarsus	Tarsometatarsus
Proximal	50%	61%	75%
Distal	50%	39%	25%

---

b) Broiler Fowls.

One bird died during anaesthesia. A pin was not placed in the femoral diaphysis of 4 birds or in the tibiotarsal diaphysis of five birds. The growth at the individual bone extremities is recorded in Table N. The average percentage of growth at the proximal and distal extremities was calculated (Table O). The average long bone growth and the average growth at each bone extremity in the S line and broiler fowls was plotted in Fig 121.

Examination of the radiographs of the sixty long bones containing metallic markers revealed dyschondroplastic type defects of endochondral ossification in ten of the bone extremities. The proportional percentage of growth at each dyschondroplastic physis relative to the total growth of that long

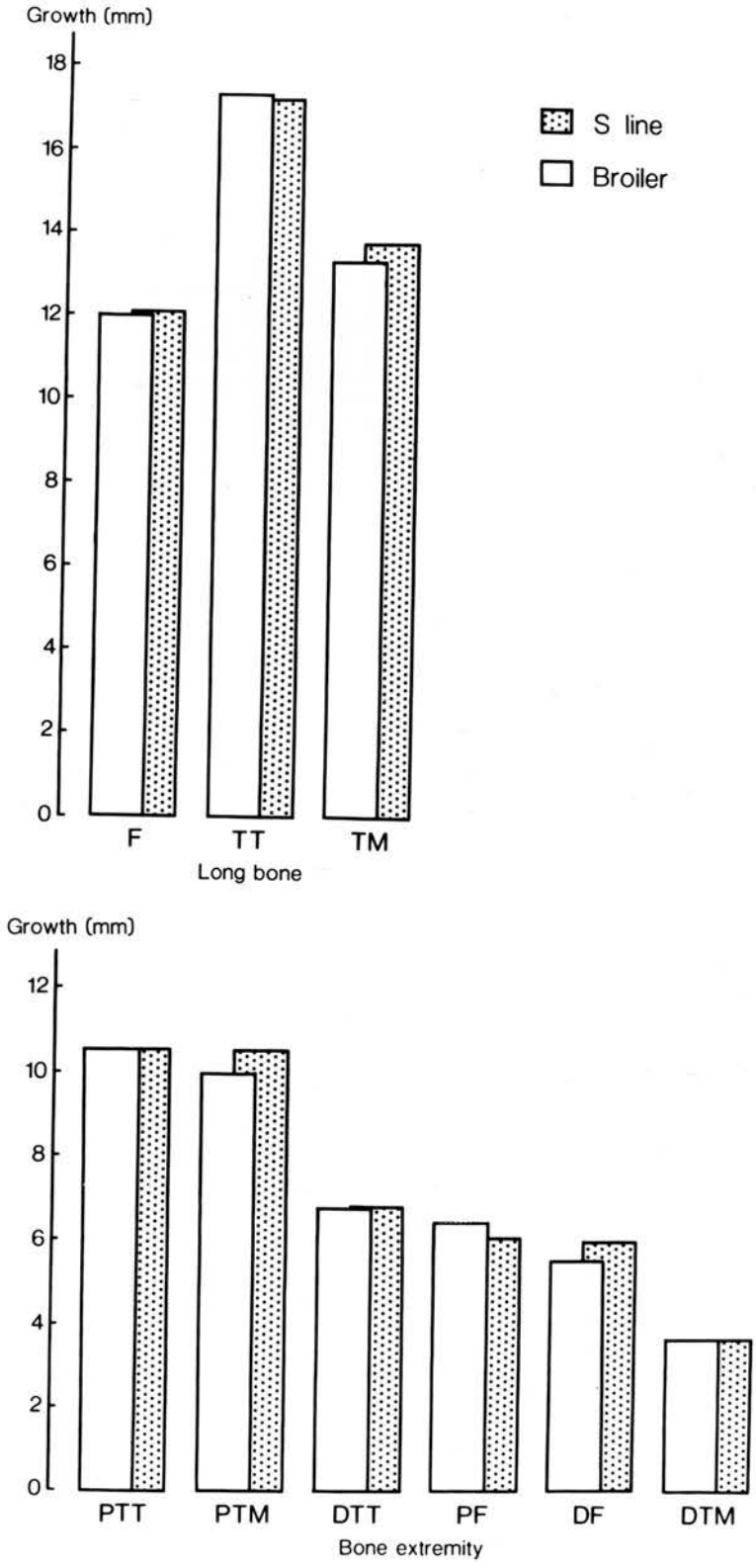


Fig 121. Long bone growth and individual bone extremity growth in S line (from 13 to 27 days of age) and broilers (from 14 to 22 days of age).

TABLE N

Growth (mm) at each bone extremity.

	Femur		Tibiotarsus		Tarsometatarsus	
Bird No.	Prox	Dis	Prox	Dis	Prox	Dis
1175	12	13	22	16	20	7
1174	15	13	25	17	23	6
1169	12.5	13.5	14	17	20	8
1181	-	-	-	-	20	6
1178	12	12	19	11	20	8
1172	15	12	-	-	23	8
NWB	12	14	-	-	19	9
4276	15	13	22	18	23	8
1168	13	14	22	17	21	8
1173	13	13	23	16	22.5	6.5
1162	-	-	23	16	20	7
1179	12	12	20	16	19	7
1171	13	13	21	15	19	8
1165	13	12	-	-	19	6
1166	-	-	18	14	17	5
1161	-	-	-	-	16	7

---

bone over the experimental period was calculated. The proportion of growth at the dyschondroplastic extremities was then compared with their predicted growth (Table P). The proportional growth rate of seven of the dyschondroplastic bone extremities was less

TABLE O.

The percentage of growth at each bone extremity in the three long bones of broiler fowl.

	Femur	Tibiotarsus	Tarsometatarsus
Proximal	53%	61%	74%
Distal	47%	39%	26%

TABLE P

The proportional growth rates of bone extremities with dyschondroplasia.

Bird No.	Dyschondroplastic Bone Extremity.	Actual Growth.	Predicted Growth.	Difference.
1168	Prox TT	56%	61%	-5
1173	Prox Femur	53%	53%	0
1169	Prox Femur	48%	53%	-5
1169	Prox TT	58%	61%	-3
1169	Prox TM	71%	74%	-3
1181	Prox TM	76%	74%	+2
NWD	Prox Femur	46%	53%	-7
4276	Prox Femur	53%	53%	0
4276	Dis Femur	47%	47%	0
4276	Prox TT	55%	61%	-6

---

than the expected growth rate. The growth rate was equal to the

expected growth rate in two physes and greater than expected in one physis.

a) Normal S line fowls.

In the S line fowls there was little inter-bird variation in the proportional rate of growth at each bone extremity. This suggests that the insertion of metallic markers into the diaphysis is a precise method of measuring the growth rate of individual bone extremities.

The growth rates of the proximal tibiotarsus and proximal tarsometatarsus were identical. The resultant proportion of growth occurring at the proximal and distal extremities of these two bones was similar to the findings of Church and Johnson (1964). They estimated that 66% and 80% of growth occurred at the proximal extremities of the tibiotarsus and tarsometatarsus respectively. In this study the corresponding estimates of growth at these two extremities were 61% and 75%. There are two factors which could account for the slightly lower estimates in the present study. A different method was used in each of the two studies to estimate the rate of growth. Also in this study proportional growth rate was estimated over a two week period, whilst Church and Johnson (1964) examined skeletons from growing fowls. The physis of the distal tibiotarsus closes before the proximal physis. The effect of earlier closure of the distal physis would be to increase the total proportion of growth at the proximal bone extremity.

Latimer (1927) proposed that there is no growth in the distal tarsometatarsus of the fowl due to the absence of a definitive

growth plate and cartilaginous epiphysis. The present study clearly demonstrates a contribution to long bone growth from the distal tarsometatarsus. The present findings are very similar to those of Roche (1963). His study of the "sites of elongation of human metacarpals and metatarsals" estimated that 20 -30% of elongation occurs at the non epiphyseal or distal extremity. The epiphyseal hyaline cartilage which underlies the articular cartilage functions as a growth zone which increases the size of the distal extremity. In the absence of a cartilaginous epiphysis and physis it is not surprising that this sub-articular zone contributes to elongation of the long bones in the fowl.

Dyschondroplasia has been correlated with growth rate in pigs (Reiland, 1974 and 1978b), cattle (Reiland et al, 1978), dogs (Olsson, 1975, 1976 and 1977) and in horses (Rejno and Stromberg, 1978), and it has been stated that predilection sites for dyschondroplasia are those where cartilage growth is most rapid (Olsson, 1982). In the broiler fowl dyschondroplasia, termed "abnormal cartilage formation", was first observed in the proximal tibiotarsus and tarsometatarsus (Leach and Nesheim, 1965), were lesions occur most frequently (Siller, 1970; Siller and Duff. 1970; Prasad at al, 1971; Reiland et al, 1977), and the most pronounced histological changes were reported (Reiland et al, 1977). The growth rates of the proximal tibiotarsus and tarsometatarsus in the broiler fowl were recognised by Riddell (1975) as being an intrinsic factor in the development of dyschondroplasia at these sites. The present study concurs with earlier reports, demonstrating that the greatest rates of long

bone growth in the broiler fowl occur in the proximal tibiotarsus and proximal tarsometatarsus, and if the incidence of dyschondroplasia is increased by faster growth rates then it is not surprising that the incidence and severity of lesions is greatest in the fastest growing bone extremities.

b) Broiler fowls

The average percentage of growth in the proximal and distal extremities of the individual long bones of broiler fowls was similar to that in S line fowls. The mean proportional growth at each bone extremity in the two studies was very similar. (Tables O and L, and Fig 121). There was a greater variation in the growth rate between the bone extremities of individual broiler fowls than there was in S line birds. The greater inter bird variation amongst broilers is probably a reflection of a less well ordered growth process.

The rearing of ad lib broiler chicks in a brooder is considered to increase the incidence of dyschondroplasia (Dewar, personal communication). The present study confirmed the development of radiologically identifiable metaphyseal defects typical of dyschondroplasia in broilers reared in brooders. The lesions were only identified radiographically and small areas of physeal thickening would not have been noted. Radiographic examination was considered to be adequate in this study as it was likely that only gross dyschondroplastic pathology would significantly affect rates of bone growth. Smaller localized lesions may result in bone deformity and would have less affect on

bone length. Rearing in brooders probably increases the incidence of dyschondroplasia by restricting activity in the birds. The space is limited in the brooder and the warmth in conjunction with ad libitum feeding would encourage lassitude.

In the dog the rate of growth in the distal radius and ulna is asynchronous. Elongation of the ulna is due principally to the distal growth plate, whereas approximately 40% of radial elongation arise from the proximal growth plate (Noser et al, 1977). When the radius and ulna were cross pinned in the growing dog there was a reduction in growth at the proximal extremity of the radius, but a compensatory increase in the growth rate of the distal radius occurred. Experimental epiphyseodesis of the growth plate in the growing rabbit resulted in increased growth from the opposite extremity (Hall-Craggs, 1968). In the present study, delayed growth in one extremity could cause a compensatory increase in the growth rate of the opposite extremity. The resulting change in the proportional growth of the two extremities would, when judged by a mid-diaphyseal marker, cause an overestimate of the reduction in growth at the dyschondroplastic extremity.

In this experiment there was a reduction in the expected growth at the majority of physes with metaphyseal defects. In man tibia vara and medial torsion of the tibia is a consequence of segmental failure of the caudomedial part of the upper tibial growth plate due to dyschondroplasia (termed: osteochondrosis) (Golding and McNeil-Smith, 1963). A similar aetiology has been suggested as a cause of limb deformity in foals (Auer, 1982a and

1982b). Earlier work in the present study demonstrated a greater variation in estimated torsion and angulation of long bones in fowls with the highest incidence of physeal abnormalities. The present investigation suggests that in fowls segmental dyschondroplasia of physes would cause a localized reduction in growth rate. Different growth rates in areas of the physis would increase mechanical forces in the physeal cartilage. The combined effects of a localized reduction in growth rate and increased mechanical forces would lead to abnormal bone torsions and angulations. The incidence therefore of torsional and angular deformities in the long bones of the fowl is likely, in part, to be due to localized disruptions of endochondral ossification.

EXPERIMENT 9:Physeal growth rate and physeal thickness

INTRODUCTION.

The thickness of physeal cartilage is considered to be an important element in the growth rate and final length of bones (Sissons, 1971; Salentijn, 1974).

In the broiler fowl, Riddell (1975d) postulated that the rapid proliferation of physeal cartilage in the proximal tibiotarsus and tarsometatarsus may exceed the rate of vascular invasion and erosion of hypertrophied cartilage. If this was the case then physeal thickness would increase markedly during periods of rapid growth. In the present study experiments 3, 4 and 5 had given the impression that physeal thickness was greater in faster growing fowls. Thicker physes have increased metabolic requirements (Yabsley and Harris. 1963). If physeal thickness increases with the rate of bone growth then their may be a point reached were the vascular supply was unable to meet the metabolic requirements of the cartilage.

The purpose of this experiment was to investigate the relationship between physeal thickness and growth rate.

MATERIAL AND METHODS.

The average rate of growth, between kills, of each long bone was calculated for ad libitum fed broilers, restricted broilers and add libitum fed S line (experiments 3, 4 and 5).

The percentage of growth occurring at each extremity of the three long bones was calculated in experiment 8. From this information the average rate of growth of the three proximal physes, between kills, could be calculated. Only proximal physes were considered from two to ten weeks of age.

The thickness of physeal cartilage was measured in each proximal bone extremity of the birds in experiments 3, 4 and 5. Measurements were made at the centre of physes in the Polymast erresin slabs. A graticule in one eyepiece of the binocular microscope was used to accurately measure physeal thickness. The average thickness of physeal cartilage in each of the three proximal bone extremities of each age group was then calculated. These measurements and calculations were repeated in all three groups of fowls (restricted broiler, ad libitum broiler and S line)

RESULTS

The thickness of physeal cartilage in each bone extremity varied little between individuals of the same age and from the same group. Physeal cartilage from different bone extremities in the same bird was thicker in faster growing physes.

The average growth rate of each bone extremity at different ages was plotted against the average physeal width in each of the three groups (Fig 122). The growth rate varied from 1.8 to 8.4mm per week. Physeal thickness varied from 0.18 - 0.84mm. The relationship between physeal thickness and growth rate over the range of growth was found to be linear. The thickness of the physis was approximately one tenth of the bone growth occurring at that extremity.

The length of the PEVs was slightly shorter than the thickness of the physis. The impression gained was that the thickness of the physis and the size of the PEVs remained proportional. The thickest physis was that in the proximal tibiotarsus of the ad libitum fed broiler.

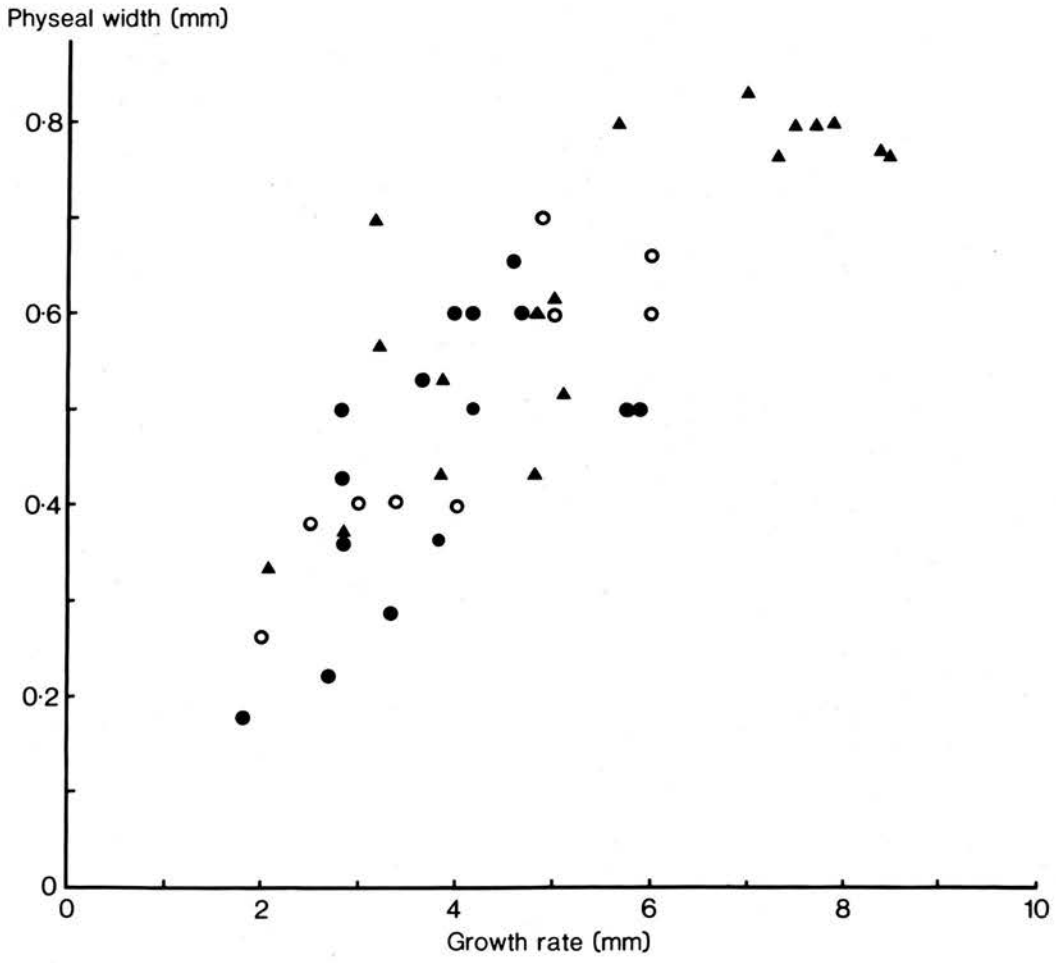


Fig 122. The average width of the physis at different growth rates in ad lib broiler (▲), restricted broiler (o) and ad lib S line fowl (●).

DISCUSSION.

The present study was carried out in normal physes with no evidence of pathological change. Gross physeal thickening occurs in some disease states, such as rickets (Lacey and Huffer, 1982; Huffer and Lacey, 1982). In the present study of normal avian physes there was a precise linear relationship between thickness and growth rate over a wide range of values. This relationship was maintained even at high growth rates, and suggests that in these physes the MVs were capable of keeping pace with the rate of physeal cartilage proliferation.

The thickness of the physis was approximately one tenth of the increase in length occurring at that physis in one week. From these figures it can be inferred that chondrocytes from the top of the physis will be moved to the bottom of the physeal cartilage in one tenth of a week, approximatly 17 hours. The time interval of 17 hours is the cycle or turnover rate of the chondrocytes. The mean cycle time of proliferating cells in the physis of man is 20 days and in the rat it is 2 days (Kember and Sissons, 1976). The more rapid cycle time in the fowl means that the effect of disruption to the physis will have a more immediate effect on endochondral ossification.

In the present study the time taken for the maturation of physeal chondrocytes and matrix remained constant at different growth rates. The response to a faster growth rate is an increased rate of division of physeal chondrocytes (Kember, 1960). In the present study the effect of a change in the rate of

division of chondrocytes was a proportional change in the thickness of the physis. From these results it can be inferred that the zones of chondrocytes in the physis will vary in thickness proportionally with growth rate. In experiments 4 and 10 of the present study clefts were reported in physeal cartilage. The incidence of physeal clefts was highest in broiler fowls. In man clefts in the physis of the femoral head are associated with weakness in the hypertrophied zone of chondrocytes (Mickelson et al, 1977), and predispose to epiphyseolysis. In the present study the clefts were in the transitional zone of chondrocytes. The increased thickness of this zone in the faster growing physes may increase the likelihood of cleft formation and subsequent epiphyseolysis.

The physeal cartilage in the fowl is maintained by PEVs, which increase in size as the cartilage becomes thicker. The maintenance requirements of a thicker growth plate will be greater. The PEVs control the concentrations of nutrients and metabolites in the cartilage matrix. The present study has already reported aberrant sizes and shapes of PEVs in the faster growing fowls. At higher growth rates the metabolic requirementof physeal cartilages may not be met in full by the vascular supply. The disruption of physeal vasculature even for a very short period of time could result in abnormal physeal cartilage, which MVs could not readily penetrate. The likelihood of such disruption would be much greater in thicker physes, with variation in the morphology of PEVs, such as was found in rapidly growing broiler fowls.

EXPERIMENT 10:Unilateral weight bearing.

INTRODUCTION.

There is a very high incidence of dyschondroplastic type lesions in the appendicular skeleton of unilateral weight bearing (UWB) broiler fowls (Duff,1986f). These lesions were frequently associated with changes in the vascular morphology of the physis. Increased functional loading has been associated with a greater incidence and severity of dyschondroplasia in a number of species. When the right foreleg of pigs was strapped to the abdominal wall, dyschondroplasia developed in the left ulna due to increased loading. Lead saddles were also strapped to the backs of growing pigs causing bilateral disruption of the ulnar metaphyses due to dyschondroplasia. The pathology was considered to be an exaggerated form of the "normal" morphology in the distal ulnae of growing pigs (Walker et al, 1966). Physeal thickening was induced in dogs by overloading one limb following triceps or archilles tenotomy in the contralateral limb (Paatsama et al, 1972). Similar pathology was reported by Grondalen and Grondalen (1974b) in overloaded physes of the growing pig. Osteochondrotic lesions were demonstrated in the limbs of lambs subjected to functional overloading (Duff, 1986a, 1986b and 1986c). Riddell (1975a) investigated the effect of overloading on dyschondroplasia in the fowl by sectioning gastrocnemius and flexor tendons. The resulting pathology contradicted other reports, because Riddell

(1975) found a lesser severity of lesions in the overloaded limb.

The present study has already elucidated the vascular morphology of bone extremities in normal broiler fowls. The purpose of this experiment was to investigate the vasculature associated with the development and repair of physeal lesions in the limbs of UWB fowls.

MATERIAL AND METHODS.

Whilst the other experimental work was being carried out a number of skeletally immature UWB broilers became available for study. This provided an opportunity to study vascular changes during the formation and repair of physeal lesions in overloaded and unloaded limbs. Fifteen of these birds were perfused and examined routinely at post mortem. The bone extremities from the pelvic limbs were embedded in resin. The Polymaster blocks containing the proximal and distal femur, proximal and distal tibiotarsus and the proximal tarsometatarsus were subsequently cut into slabs and examined. Fifty slabs were re-embedded in Polymaster resin for subsequent histological examination.

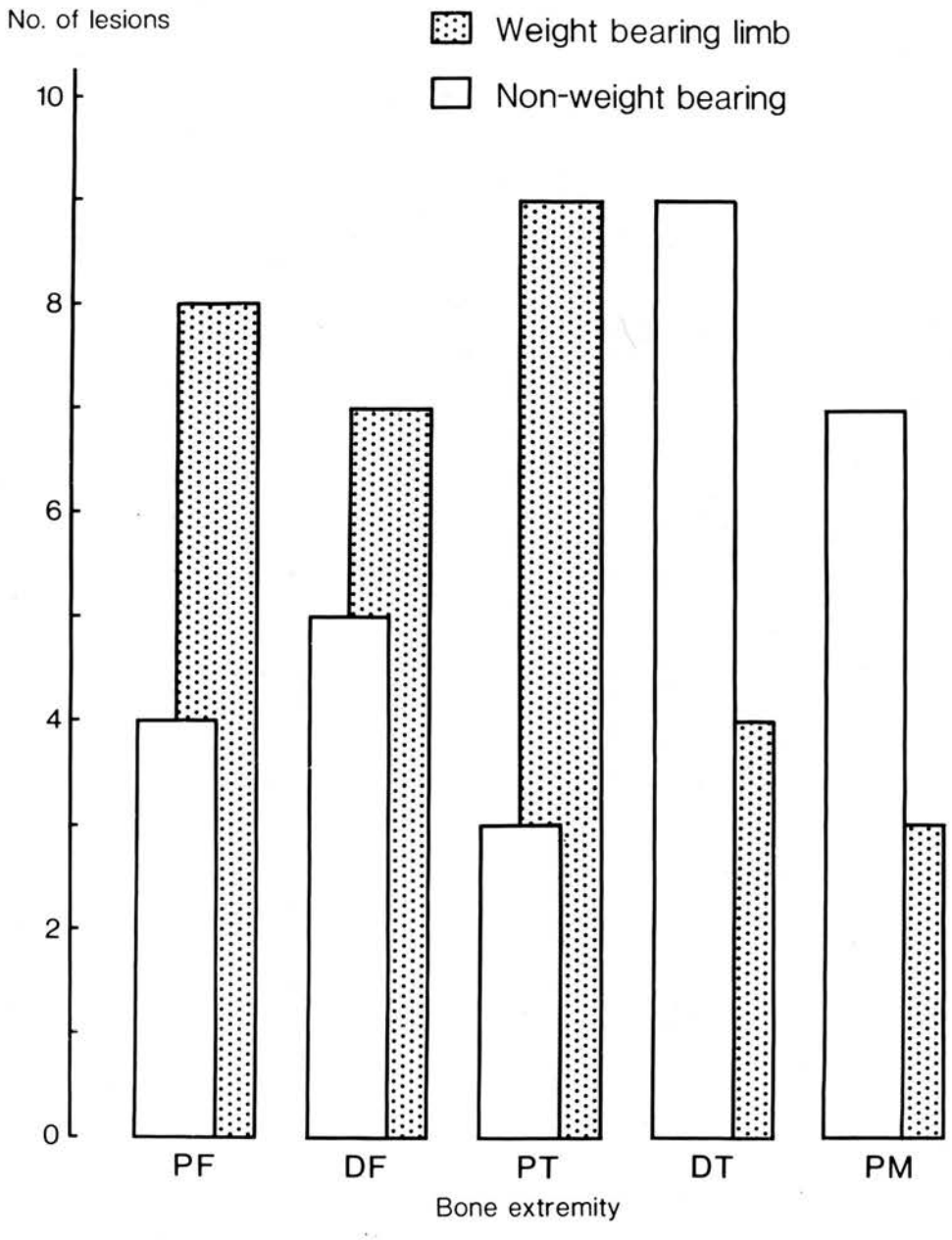


Fig 123. Histogram demonstrating the number of lesions occuring in each long bone extremity of birds with a unilateral weight bearing stance. The weight and non-weight bearing limb are considered separately.

RESULTS.

Of the 150 bone extremities examined there were lesions in 59. 31 of the lesions occurred in the weight bearing limb and the remaining 28 in the non-weight bearing limb. In nine of the UWB fowls the left limb was weight bearing, whilst in the other six it was the right limb which was load bearing.

The distribution of lesions varied between limbs (Fig 123). In the weight bearing limb the lesions tended to occur in the femur and proximal tibiotarsus. In the non-weight bearing limb the distal tibiotarsus and proximal tarsometatarsus most commonly showed pathological changes.

Proximal femur (weight bearing).

There were lesions in eight extremities.

The most frequent lesion, which occurred in four of the specimens, was a delay in MV invasion of the craniomedial femoral head. This occurred in conjunction with a local absence of EVCs and PEVs (Fig 124). In two of the specimens the craniomedial femoral head was being revascularised by EVCs descending from the capital femoral ligament. Histological sections confirmed the presence of occluded EVCs (Fig 125) and revascularising vessels. The occluded EVCs showed patchy staining, cellular debris and pyknotic nuclei.

In three proximal femurs there were dyschondroplastic lesions of the mid-femoral head with a delay in MV invasion of physeal

Fig 124. The femoral head from a 3 week old UWB broiler. EVCs and PEVs are absent from the cranio-medial femoral head. A "starburst" of EVCs is descending from the teres ligament to revascularise the avascular cartilage. 1mm slab x16.

Fig 125. This section is cut from the slab shown in fig 124. There are occluded EVCs (arrowed) in the epiphyseal hyaline cartilage. MGT x20.

cartilage. The thickening of physeal cartilage was caused by an increase in the number of prehypertrophied chondrocytes. In these three lesions PEVs were present in the physeal cartilage,and in one lesion the PEVs were elongated.

In one of the specimens there was a generalised disruption of endochondral ossification and advancement of MVs into the physeal cartilage. The medial physis was thickened. Throughout the femoral head there was an absence of EVCs and PEVs. Histological sections of the femoral head demonstrated the occlusion of cartilage canals. The metaphysis contained a large cone of cartilage. The cartilaginous epiphysis was revascularising from the capital femoral ligament. Some of the EVCs, from the caudal perichondrial ring, formed elongated PEVs which crossed the physis into the avascular mass of retained cartilage (Fig 128).. One of these elongated PEVs divided extensively to produce a "starburst" of vessels eroding into the avascular cartilage (Fig 126). The retained physeal cartilage around the transphyseal PEVs was hypertrophied and the matrix calcified.

There were clefts in the physes of two of the femoral heads. Masson Goldner trichrome stained sections demonstrated that the clefts contained amorphous material and cellular debris. The clefts occurred in the craniomedial physis where there was also an increase in physeal thickness and an absence of PEVs. In one of these cases, the avascular cartilaginous epiphysis and physis contained clefts and areas of necrosis (Fig 129). In another example, with severe dyschondroplasia of the proximal head, the PEVs in the centre of the physis were elongated and extended into

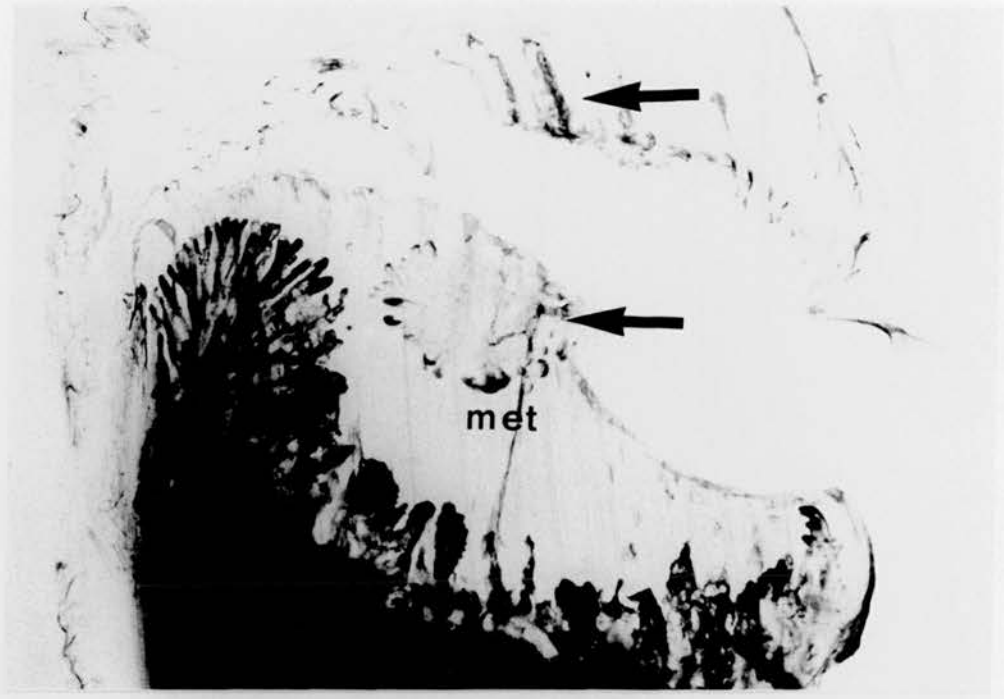


Fig 126. The proximal femur of a UWB broiler. There is severe dyschondroplasia and epiphysiolysis of the femoral head. A transphyseal PEV (arrowed) forms a "starburst" of vessels as it attempts to revascularise the mass of cartilage occupying the metaphysis. 1mm slab x10.

Fig 127. The proximal femur from a 6 week old UWB broiler. There are many elongated transphyseal PEVs with lateral branches revascularising the retained physeal cartilage. 1mm slab x10.

the avascular cartilage where they divided (Fig 127).

Proximal femur (non-weight bearing).

There were lesions in four extremities.

In three extremities there was delayed MV invasion of the craniomedial femoral head, with an associated accumulation of prehypertrophied chondrocytes. The craniomedial femoral head was poorly perfused by EVCs and histological sections demonstrated the presence of occluded vessels. EVCs from the capital femoral ligament revascularised the cartilaginous epiphyses in all three femoral heads. In the centre of one femoral head the physeal cartilage was thickened and PEVs elongated. The physeal thickening was due to an increase in the numbers of prehypertrophied chondrocytes.

Distal femur (weight bearing).

There were lesions in seven extremities.

Lesions involving the physis and metaphysis occurred in the medial condyle of four specimens, in the lateral condyle of another and in both condyles of two cases. In one of the birds, where both condyles were involved, there was a large dyschondroplastic mass which occupied most of the metaphysis. In the other specimens the lesions were a localized, usually peripheral, thickening and delayed MV invasion of the physeal cartilage.

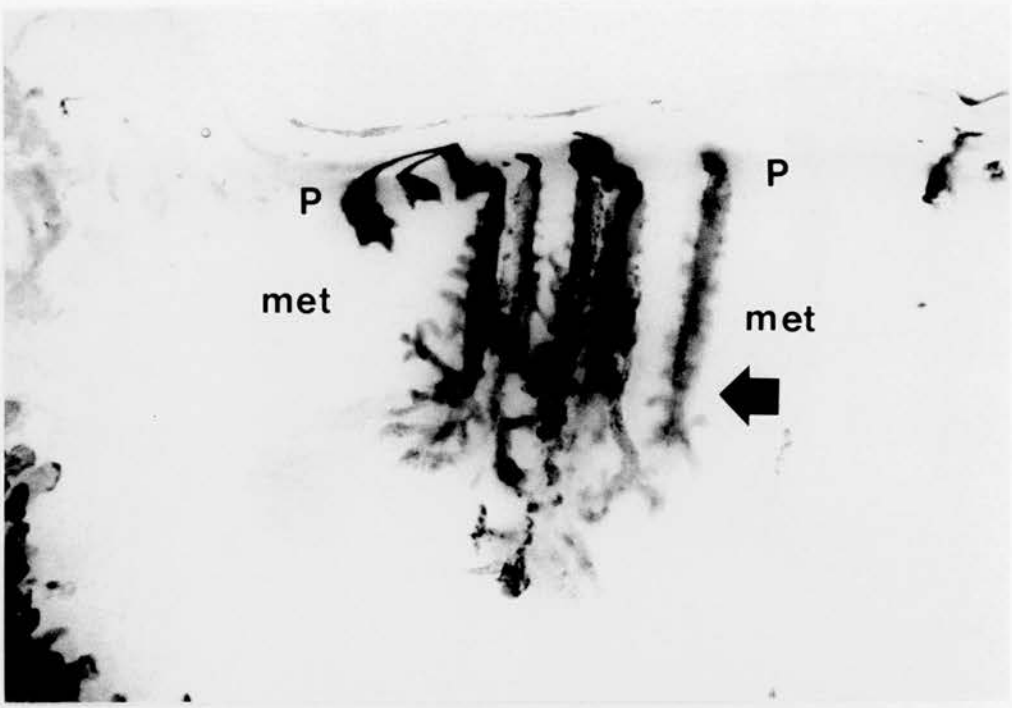


Fig 128. The proximal femur from a 5 week old UWB broiler. The entire metaphysis is occupied by avascular cartilage. Transphyseal PEVs (arrowed) are attempting to revascularise the lession. 1mm slab x16.

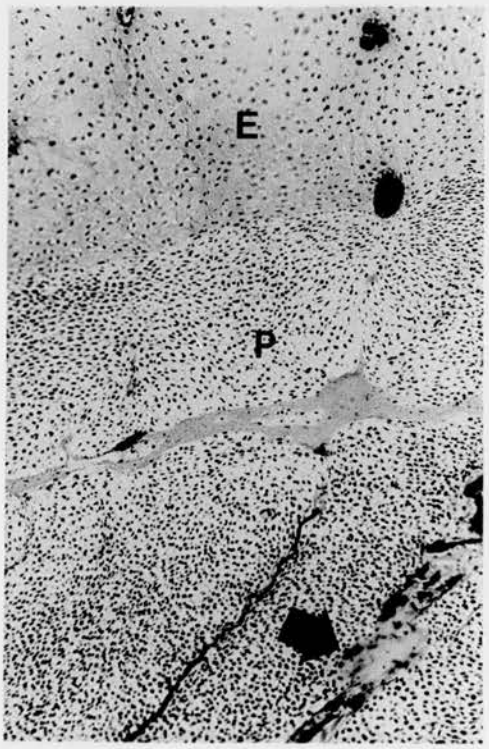


Fig 129. The femoral head from a 5 week old UWB. There is a necrotic (arrowed) in the physis of the cranio-medial physis. PEVs and EVCs are occluded. MGT x20.

Lesions in the medial aspect of the distal femur were all similar. The medial metaphyses were flattened in profile because of delayed MV invasion of the physes (Fig 130). The physes were thickened by an increase in the thickness of the prehypertrophic zone of chondrocytes. EVCs originating from the medial ICRVs were few in number or absent. MVs underlying the thickened physes were widened and blunt ending. In all the specimens there was a varying increase in the width of the metaphysis. This caused a medial bulging of the metaphysis where the MVs were extending around the lesion. In one specimen there were more extensive principal EVCs than normal supplying the medial condyle. The principal EVCs supplied an area which normally derived its vasculature from medial ICRVs. Histological sections from the medial extremities contained scars of occluded PEVs and EVCs (Fig 131). In the cartilage matrix directly adjacent to the occluded vessels there was chondrocyte cell death. PEVs which remained patent in the thickened physis were frequently elongated and they were associated with local chondrocyte hypertrophy and cartilage matrix calcification.

In the two lesions in the lateral periphery of the physis there was also a pattern of occluded vessels and a build up of prehypertrophied chondrocytes, with delayed MV invasion of the physeal cartilage. One of the distal femurs had extensive clefts in the physeal cartilage parallel with and close to the epiphyseal hyaline cartilage. The clefts contained degenerating erythrocytes. The MVs below the thickened physis were characteristically widened and blunt ending. In the other lateral

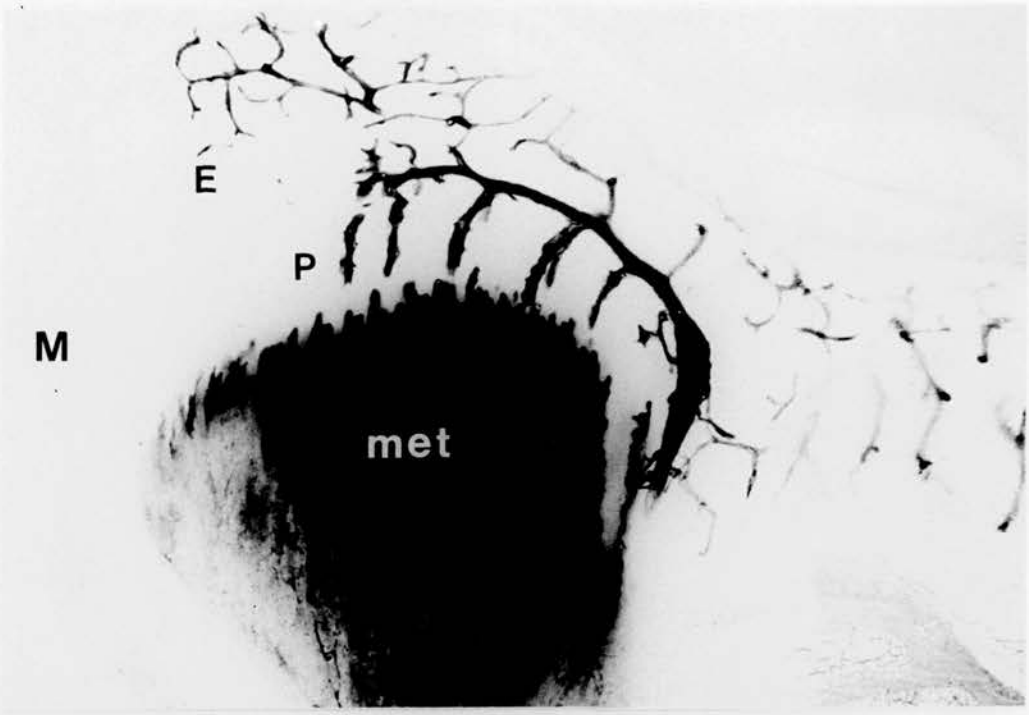


Fig 130. The distal femur from a 4 week old UWB broiler. EVCs and PEVs are absent from the medial condyle. 1mmm slab x16.

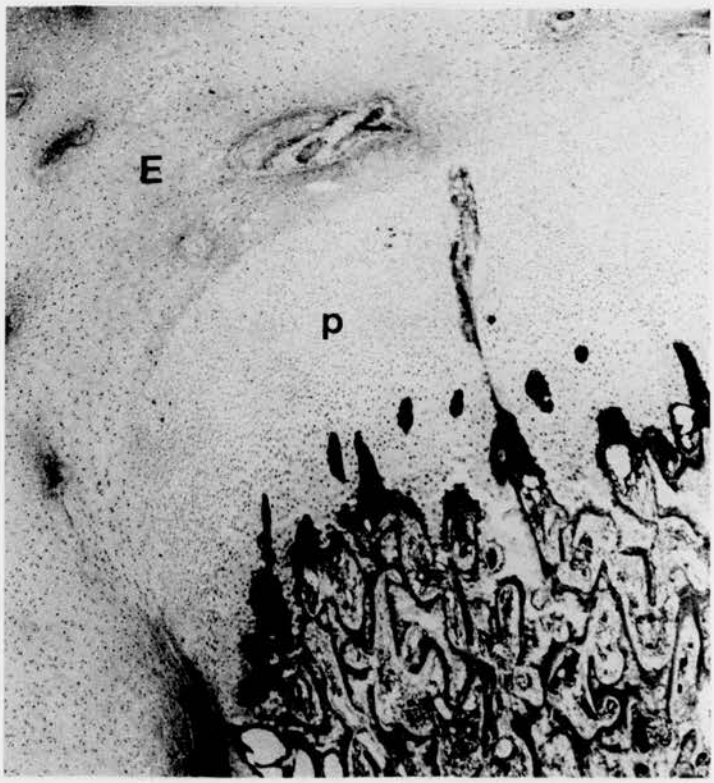


Fig 131. A section cut from the slab shown in fig 130. The physis is thickened due to a build up of prehypertrophied chondrocytes. Above the area of physeal thickening there are occluded EVCs and PEVs. MGT x40.

physeal lesion there was a grossly misshapen metaphysis caused by MVs branching laterally around the thickened physis. There were short PEVs entering the thickened physis.

In the metaphysis of the distal femur which contained the large cone of physeal cartilage, elongated PEVs had penetrated the retained cartilage core (Fig 132). These elongated PEVs extended into the cartilage cone from the intercondylar physis. The condylar PEVs however were of normal length. Perichondrial ring derived MV-type vessels penetrated and branched into the cartilaginous cone that occupied the metaphysis (Fig 133). Both the perichondrial derived MVs and the elongated PEVs were associated with localized chondrocyte hypertrophy and matrix calcification. There was a complete absence of chondrocyte hypertrophy and matrix calcification around the "normal" sized PEVs of the condyles. The MVs that were attempting to erode the retained physeal cartilage were grossly thickened, blunt ending and not associated with chondrocyte hypertrophy (Fig 134 and 136).

Distal Femur (non-weight bearing)

There were lesions in five extremities.

The type of lesions which occurred in the non-weight bearing limb were similar to those noted above. There were however no physeal clefts in non-weight bearing distal femurs.

In three specimens, thickening of the medial physis occurred in association with occluded EVCs and PEVs which originated from the medial ICRVs. In the lateral physis of one specimen, delayed

Fig 132. The distal femur from a 4 week old UWB broiler. A large mass of avascular cartilage occupies the metaphysis. The PEVs are normal or elongated. There is penetration of the abnormal cartilage by MV like vessels derived from perichondrial ring (arrow). 1mmm slab x16

Fig 133. This section is prepared from the slab shown in fig 132. The vessels derived from the perichondrial ring have the appearance of MVs as they erode the avascular cartilage. MGT x20.

MV invasion of a thickened physis was present. There were occluded EVCs and PEVs and a build up of prehypertrophic chondrocytes.

The bird with an extensive dyschondroplastic defect in the metaphysis of the distal femur of the weight bearing limb had a similar lesion in the non-weight bearing limb.

Proximal tibiotarsus (weight bearing).

There were lesions in nine extremities.

The most frequent abnormality, which occurred in six limbs, was a thickening in the physis of the lateral tibiotarsus adjacent to the fibula. There was a delay in MV invasion of the physeal cartilage. The MVs were thickened and blunt ending and the metaphysis was bulging laterally below the cartilage defect. In all but one specimen PEVs were present in the thickened physeal cartilage and were either elongated or of normal length. Despite the presence of PEVs there was delayed calcification of the cartilage matrix in thickened physes (Figs 136a and 136b). The lateral physis and cartilaginous epiphysis of one proximal tibiotarsus was poorly perfused and histological sections demonstrated vascular occlusion of PEVs and EVCs in this region.

In one proximal tibiotarsus, a lesion was noted in the physis below the epiphyseal ossification centre. The EVCs and PEVs below the EOC were occluded. There was delayed MV invasion of a slightly thickened physis and disrupted endochondral ossification in the adjacent periphery of the EOC. There were small clefts in

Fig 134. This section is also prepared from the slab shown in fig 132. The MVs (arrowed) are from the base of the abnormal cartilage. The MVs are widened and blunt ending they are failing to penetrate the avascular cartilage and bring about endochondral ossification. MGT x20.

Fig 135. The MVs (arrowed) are from the periphery of the base of the retained cartilage mass shown in fig 132. The MVs are irregular and disorganised as they attempt to penetrate the abnormal cartilage. MGT x20.

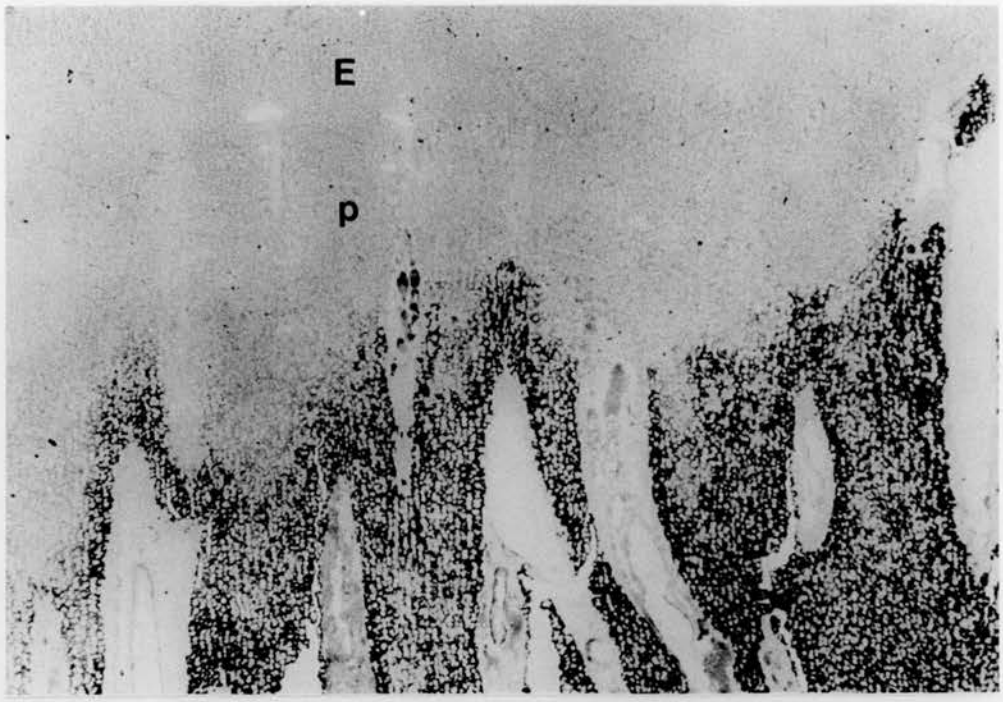


Fig 136a. The lateral condyle fom a 5 week old broiler. There is thickening of the physis due to an accumulation of prehypertrophiad condrocytes. PEVs are absent from the thickened physis. The physeal cartilage has failed to calcify. Von kossa x20.

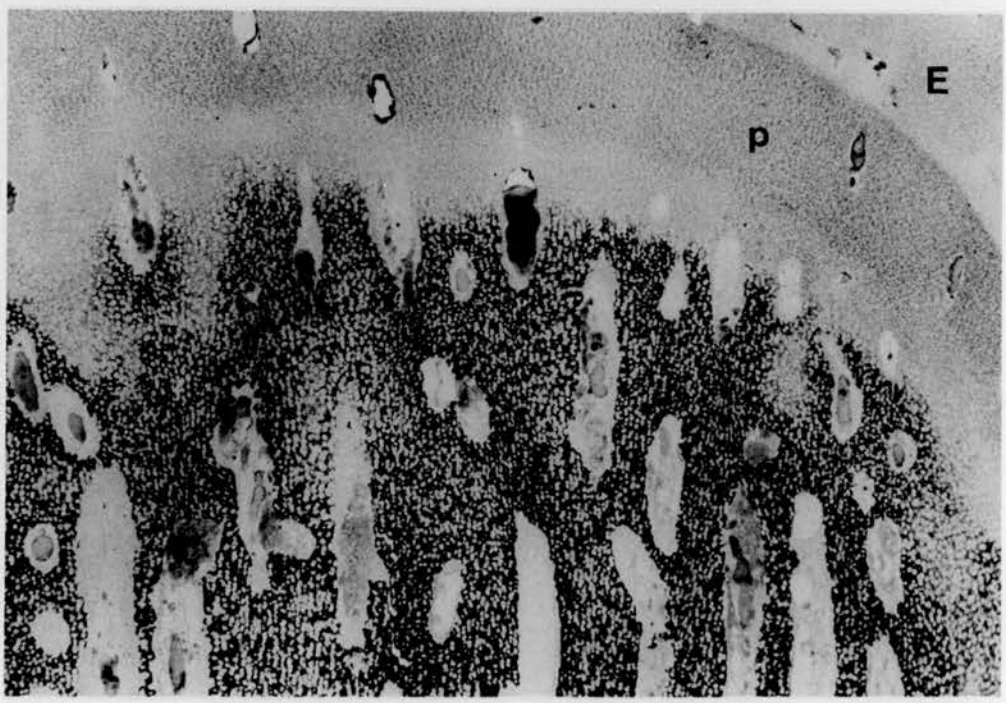


Fig 136b. The medial condyle from the same speciman as in fig 136a. The physis is normal and PEVs are present. Von kossa x20.

the physis below the EOC, traversing occluded PEVs. Adjacent EVCs were advancing into, and revascularising, the periphery of the lesion.

In three of the proximal tibiotarsi the entire physis/metaphysis was abnormal. There was a generalised increase in physeal thickness due to a build up of prehypertrophied and hypertrophied chondrocytes. The MVs were widely spaced and of increased diameter. Two of these bone extremities contained a large mass of degenerating prehypertrophied chondrocytes occupying the centre of the presumptive metaphysis (Fig 137a). Elongated PEVs some of which had branches extending down into the abnormal physeal cartilage. Around these extensive PEVs chondrocyte hypertrophy and calcification of the matrix occurred (Figs 137b and 137c). MV-type vessels, emanating from the perichondrial ring, radiated into the abnormal cartilage. These MVs were also associated with localized areas of chondrocyte hypertrophy and matrix calcification (Fig 137d). Grossly expanded MVs were formed by the blunt ending already thickened MVs that were failing to penetrate the mass of cartilage. These large mushrooming MVs, as they eroded the degenerating prehypertrophied chondrocytes, caused the margin between the MVs and the cartilage to assume a scalloped appearance. These large mushrooming MVs were associated with an absence of chondrocyte hypertrophy and matrix calcification (similar to those in Figs 134 and 135). Normal endochondral ossification had only resumed where the MV supply below physes had become re-established.

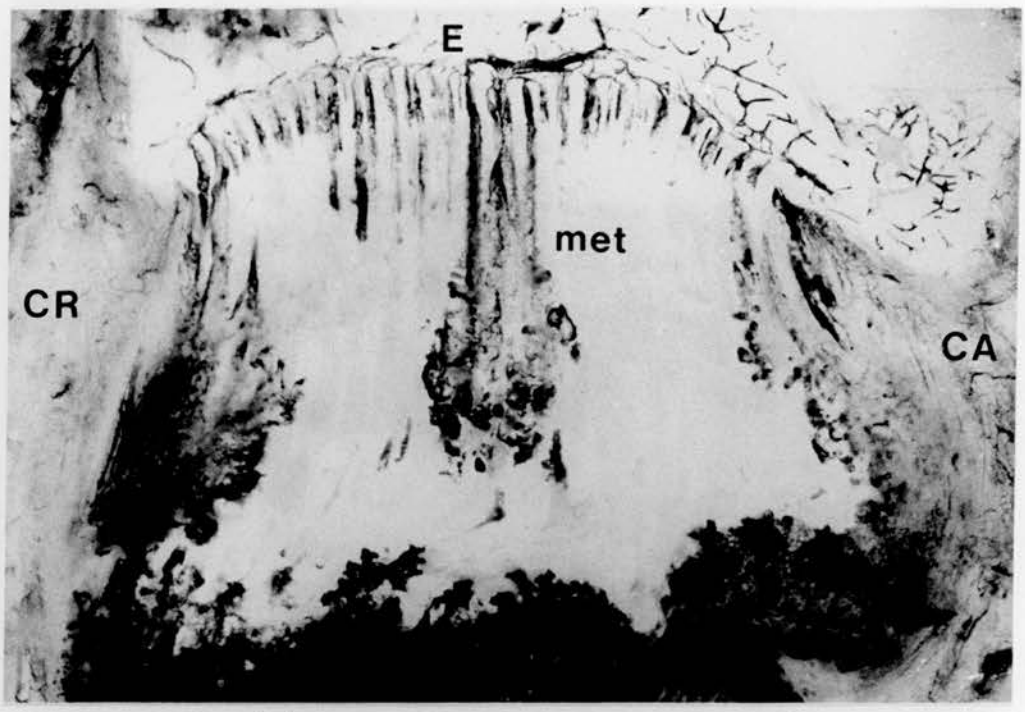


Fig 137a. The tibiotarsus from a 4 week old UWB broiler. A large mass of retained cartilage is present in the proximal metaphysis. The centre of the retained cartilage is penetrated by transphyseal PEVs. 1mm slab x10.

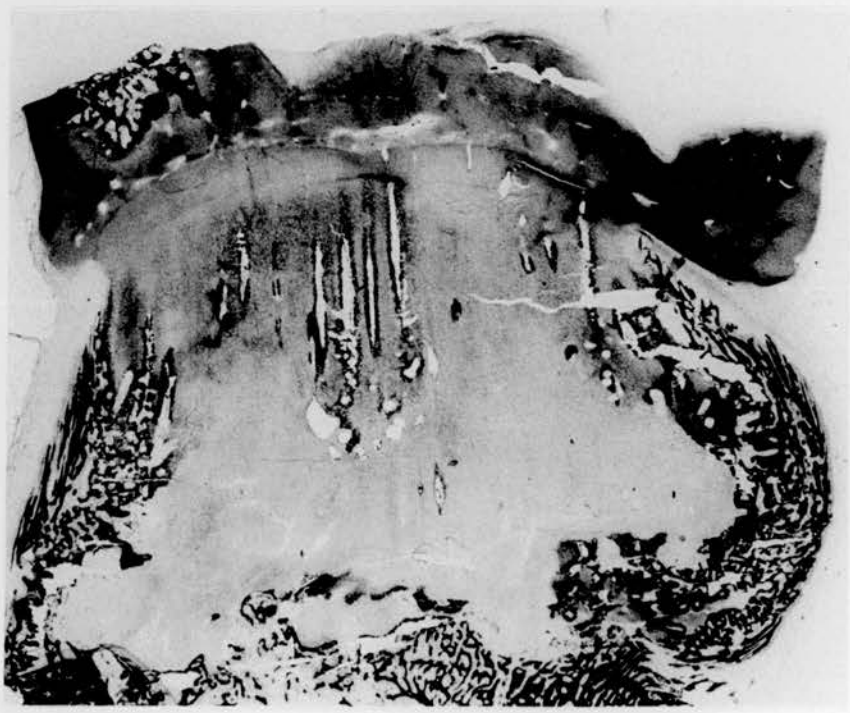


Fig 137b. A section cut from the slab shown in fig 137a. There is calcification of the cartilage around the transphyseal PEVs. Von kossa x10.

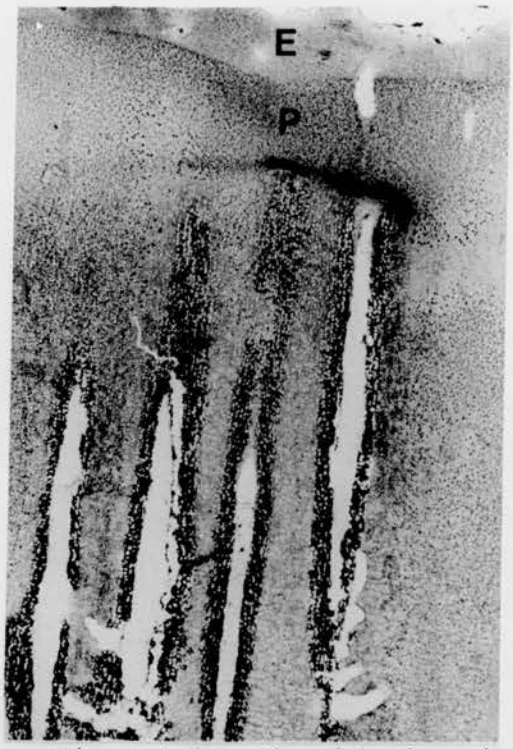


Fig 137c. A section cut from the slab shown in fig 137a. There is hypertrophy of the chondrocytes and calcification of the matrix adjacent to the transphyseal PEVs. Von kossa x25.

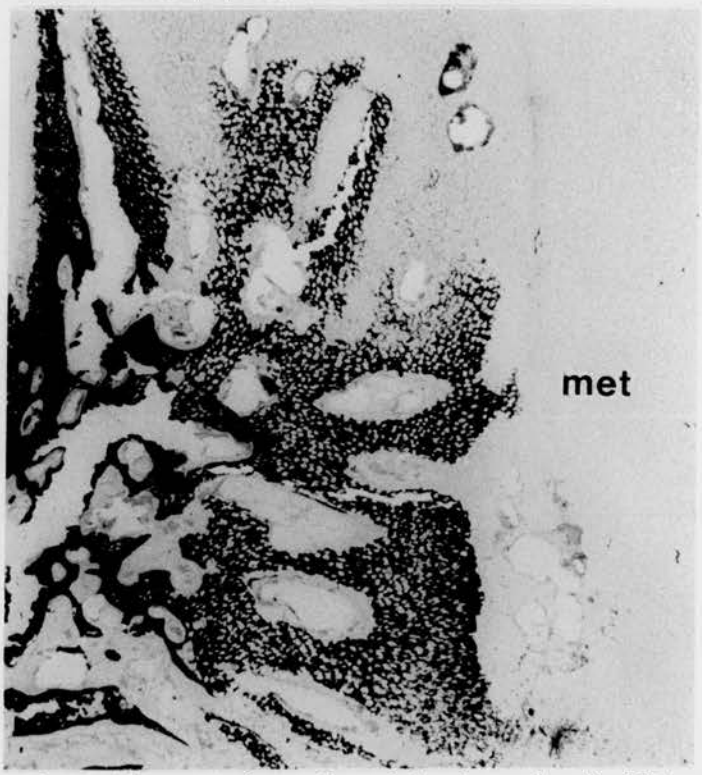


Fig 137d. A section cut from the slab shown in figl37a. There is hypertrophy of the chondrocytes and calcification of the matrix in the cartilage associated with the perichondrial derived MVs. Von kossa x20.

Proximal tibiotarsus (non-weight bearing)

There were lesions in three extremities.

The two birds with large dyschondroplastic masses in the weight bearing proximal tibiotarsi had similar gross lesions at the same site in the non-weight bearing limb. In one of these specimens, an enlarged PEV adjacent to the physeal defect anastomosed with the metaphyseal circulation.

The only other lesion to occur at this site was a medial thickening of the physis. There was an increased number of prehypertrophied chondrocytes associated with delay in MV invasion of physeal cartilage. PEVs were of a normal length.

Distal Tibiotarsus (weight bearing)

There were lesions in four extremities.

Three lesions occurred in the lateral physis and one medially.

The medial lesion was a narrow cleft, perpendicular to the direction of growth, running through the physis. The cleft containing haemorrhage traversing the prehypertrophied chondrocytes.

The three lateral lesions were all forms of dyschondroplasia. Two of the lesions were small, and showed a localized absence of EVCs and PEVs with physeal thickening due to prehypertrophied chondrocytes and delayed MV invasion. The MVs were of increased diameter and blunt ending.

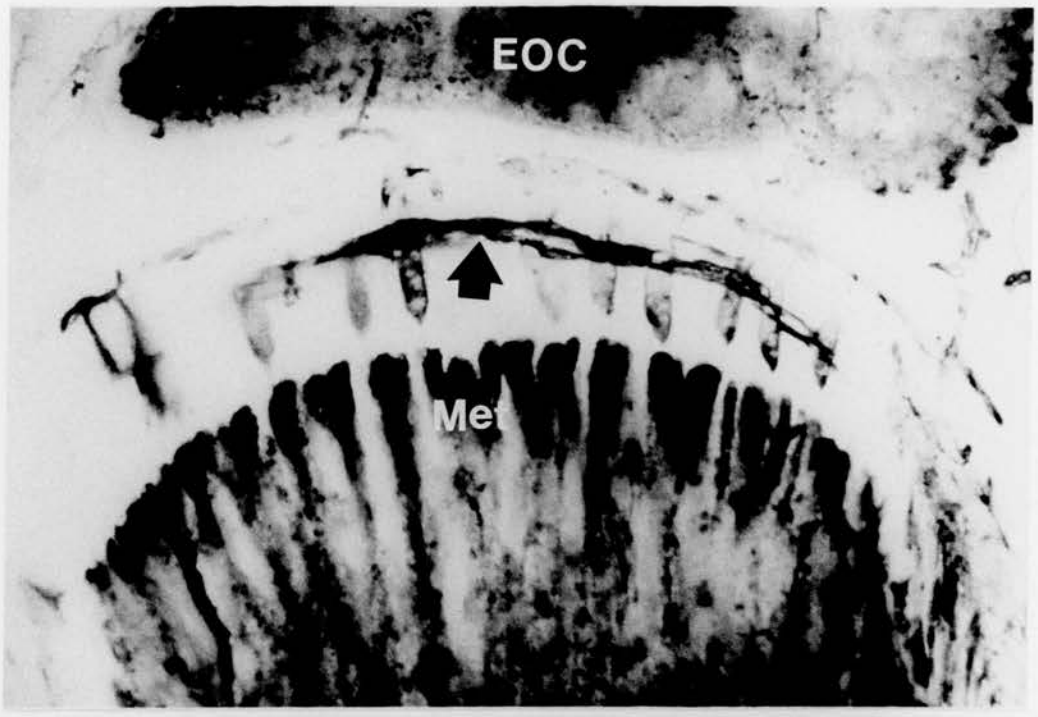


Fig 138. The distal tibiotarsus from a 4 week old UWB broiler. There is a cleft containing haemorrage (arrowed) in the physeal cartilage. 1mm slab x25.

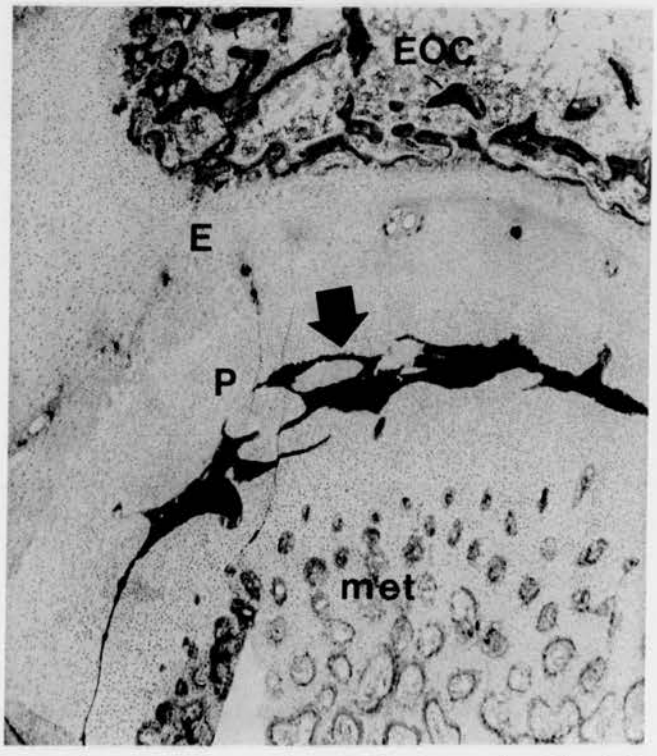


Fig 139. A section cut from the slab shown in fig 138. The cleft (arrowed) contain haemorrage and cellular debris. MGT x40.

In the third distal tibiotarsus with a lateral lesion there was a large dyschondroplastic mass of retained physeal cartilage. Across the width of the physis there were PEVs of a normal length. From the lateral perichondrial ring MV-type vessels were invading the mass of prehypertrophied cartilage. These MV-type vessels were distinct and separate from the MVs at the base of the lesion. The MVs below the cartilage mass were enlarged and blunt ending, and they were dividing in an attempt to branch around the lesion.

Distal tibiotarsus (non-weight bearing)

There were lesions in nine extremities.

The lateral condyle of five specimens contained lesions.

Four of the five lateral physeal lesions were areas of delayed MV invasion into a physis thickened by a build up of prehypertrophied chondrocytes. Below the physis the MVs were reduced in numbers, irregular and divided around the base of lesions. PEVs were present in all the physes and were frequently elongated, extending into retained physeal cartilage. There was a degree of lateral metaphyseal bulging associated with lesions.

In two distal tibiotarsi with lateral physeal lesions there were large clefts in the physeal cartilage (Fig 138). The clefts were perpendicular to the direction of growth and contained areas of haemorrhage and cellular debris (Fig 139).

Two specimens had medial lesions and in one case there was severe dyschondroplasia with gross thickening of the entire physis. Both medial lesions involved a peripheral thickening of

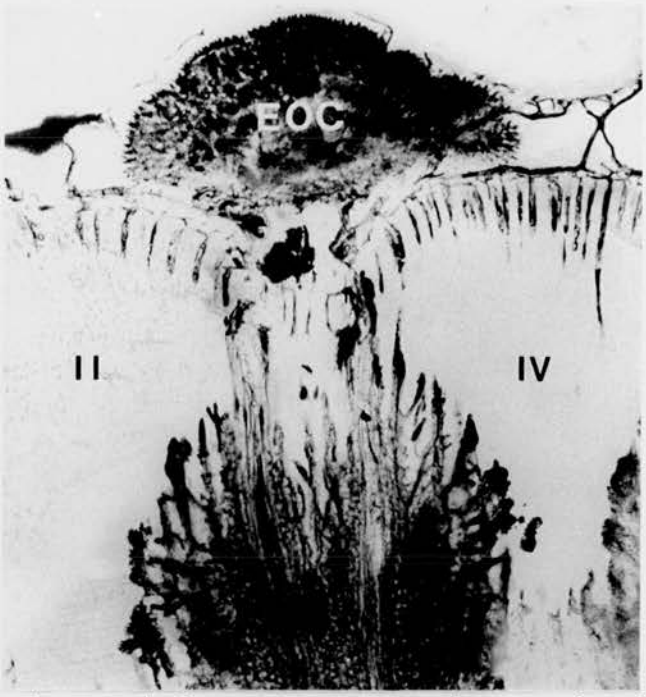


Fig 140. The proximal tarsometatarsus from a 6 week old UWB broiler. There is symmetrical cartilage retention in the metaphysis of the II and IV metatarsi. The majority of the PEVs are normal in length, although occasional elongation occurs. 1mm slab x10.

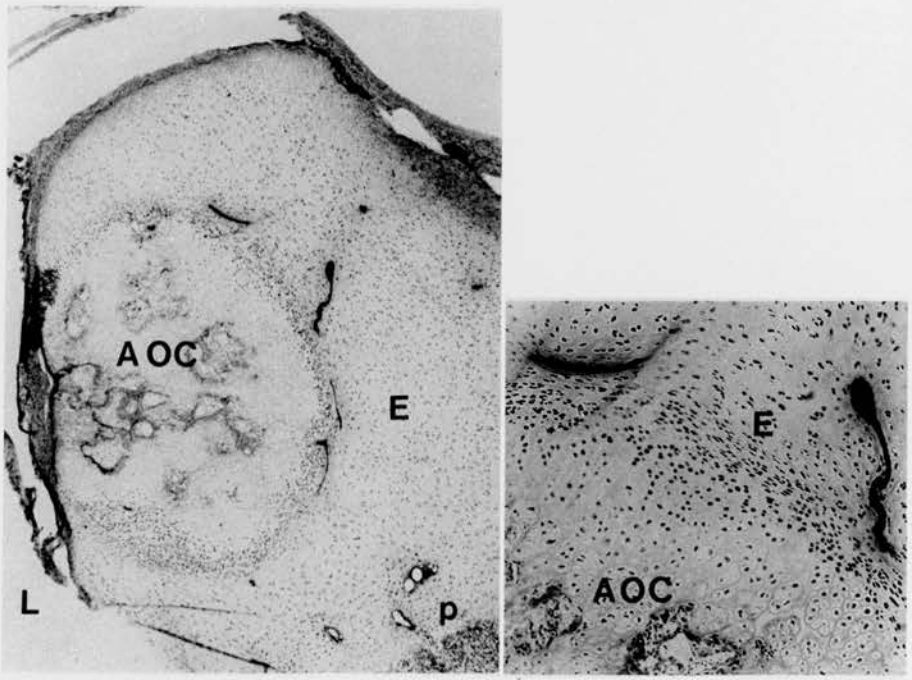


Fig 141 The proximal tarsometatarsus from a 7 week old UWB broiler. There is an accesory ossification centre (AOC) in the epiphysis, deep to the site of attachment of the medial collateral ligament. MGT x10.
(Insert: There are clefts in the cartilaginous epiphysis surrounding the accesory EOC.MGT x30.)

the physis and local absence of PEVs and EVCs. In both birds there was revascularisation by EVCs from the medial ICRVs.

The distal tibiotarsus with a large dyschondroplastic defect showed enlarged thickened MVs penetrating the lesion some of which were the mushrooming type previously described. There were also elongated PEVs with lateral branches penetrating deeply into the abnormal cartilage. The physis contained many PEVs of normal length. There was no chondrocyte hypertrophy associated with "normal" PEVs. There was however chondrocyte hypertrophy and matrix calcification around the elongated PEVs and also in the cartilage which was being eroded by MVs derived from the perichondrial ring.

Proximal Tarsometatarsus (weight bearing)

There were lesions in four bone extremities.

In two of the physes there was a thickening of the lateral physis. Elongated PEVs extended into the thickened physes which contained increased numbers of prehypertrophied chondrocytes.

In one bone extremity there were symmetrical dyschondroplastic defects in the medial and lateral metaphyses (Fig140). The majority of PEVs were of normal length although at the periphery of lesions some PEVs were elongated. Distally, MVs which penetrated the abnormal cartilage were grossly enlarged.

Proximal Tarsometatarsus (non-weight bearing)

There were lesions in seven bone extremities.

Lateral luxation of the gastrocnemius tendon had occurred in four birds, and in three of these the medial cartilaginous epiphysis and articular surface was grossly misshapen. The periphery of the medial physis was increased in thickness, and penetrated by elongated PEVs. In two of the birds, without lateral luxation of the gastrocnemius tendon, similar lesions of physeal thickening in the medial physis were also noted.

Medial luxation of the gastrocnemius tendon had occurred in one hock joint. Haemarthrosis of the joint was seen at post mortem examination. The resin slabs revealed a cleft containing haemorrhage which extended through the physeal cartilage perpendicular to the direction of growth.

The three metatarsi (II, III and IV) of one proximal tarsometatarsus contained masses of degenerating, and prehypertrophied chondrocytes. Although the majority of the PEVs were of normal length, occasional elongated PEVs descended into the retained cartilage. Elongated PEVs were associated with focal chondrocyte hypertrophy and matrix calcification. MVs derived from the perichondrial ring and metaphysis were eroding and advancing into the periphery of the lesion.

There was a large dyschondroplastic mass of cartilage in one lateral metatarsus (Met IV). The lesion was identical to those occurring in all three metatarsi of the bird in Fig 140, although the other two metatarsi were normal.

In the cartilaginous epiphysis of one specimen there was an accessory ossification centre adjacent to the attachment of the

medial collateral ligament (Fig 141). There was no contact between the normal EOC, in the centre of the cartilaginous epiphysis, and the accessory EOC. The accessory EOC was increasing in size by endochondral ossification, and obtained a vascular supply from medial ICRVs. There were clefts in the cartilaginous epiphysis adjacent to and contiguous with the accessory EOC (Fig 141). The clefts contained haemorrhage and cellular debris.

DISCUSSION.

The left limb in the majority of fowls in this study was load bearing. the right limb having a greater angular deformity. A similar pattern of development of UWB in fowls was reported by Duff (1986f). The greater frequency of UWB by the left limb, and the development of abnormal angulation in the contralateral limb complements earlier reports suggesting a pattern of limb dominance may occur in the fowl (Duff and Thorp, 1985a and 1985b).

Pressure across the physis of the rabbit causes thickening due to an accumulation of prehypertrophied chondrocytes (Trueta and Trias, 1961). The pressure was considered to cause interference to the metaphyseal blood supply. A plastic insert was placed in the physeal/metaphyseal junction of the growing fowl (Riddell, 1975a). There was a failure of MV penetration and physeal thickening occurred due to an accumulation of "dyschondroplastic" type cartilage. Riddell (1975a) proposed that dyschondroplasia was due to the failure of MV penetration of physeal cartilage.

When the fore leg of the pig was overloaded in conjunction with growth hormone therapy there were more pronounced "osteochondritis" like changes in the humeral condyle than with overloading on its own (Paatsama et al, 1975). Fowls with a UWB stance have a greater frequency of lesions in the load bearing limb (Duff, 1986f). In studies of fowls with asymmetrical load bearing, caused by angular limb deformities, dyschondroplasia occurs most frequently in the overloaded limb (Duff, 1984a; Duff

and Thorp, 1985b). In the present study the greatest frequency of lesions in the load bearing limbs was in the femur and proximal tibiotarsus. The growing broiler is susceptible to dyschondroplasia and a UWB stance would be expected to result in more extensive lesions than in slower growing fowls. Indeed overloading had apparently increased the extent and incidence of dyschondroplasia in these susceptible fowls.

The disturbance of normal growth in one growth plate may lead to abnormal stress in another growth plate resulting in more deformity (Reiland et al, 1978a). The majority of the lesions in the non load bearing limb were in the bone extremities of the hock. Dyschondroplasia in one of the bone extremities in the hock may have induced, through abnormal joint loading, dyschondroplasia in the other extremity. The development of dyschondroplasia in the proximal tarsometatarsus and distal tibiotarsus may have been responsible for the development of aberrant bone angulation and torsion culmanating in luxation of the gastrocnemius tendon. This would have resulted in greater joint deformity and would also explain the high incidence of dyschondroplastic lesions in these two bone extremities. Normal bone form will only develop in the presence of normal function (Lanyon and Bourn, 1979). There will be a point in the development of abnormal bone angulation, induced by dyschondroplasia, where the resulting deformity prevents normal function. At this point, because of the absence of normal function, there may be a rapid accentuation of the bone deformity.

In the growing pig clefts have been reported in physeal cartilage of the femoral head. These clefts were considered to be

early stages of epiphyseolysis and epiphyseal separation, the occurrence of which would be increased by traumatic forces (Bhatnagar et al, 1981). Epiphyseolysis in the pig occurs spontaneously in animals approaching skeletal maturity (Duthie and Lancaster, 1964; Cunningham, 1966 and Hoorens et al, 1966). Hamilton et al (1978) reported epiphysiolysis in the femoral head of calves caused by trauma during parturition. In rats, shear cracks (clefts) were produced in vivo using 50% of the force required to produce epiphyseolysis (Bright et al, 1974), and there was a site specific distribution of cleft formation (Bright and Elmore, 1968). Physeal clefts have also been reported in lambs, and were associated with epiphyseolysis (Uhthoff, 1982; Duff, 1986a, 1986b and 1986c). The present study augments earlier reports of physeal clefts in the fowl (Riddell et al, 1983; Duff, 1986a). Clefts in physeal cartilage of the broiler fowl were considered to be a consequence of repeated minor trauma (Duff and Randall, 1986). Femoral head separation, which has been reported by Meens and Litjens (1978) and Riddell (1981), is probably a form of epiphyseolysis in the fowl. Clefts have also been reported in physeal cartilage of fowls with windswept deformities (Duff, 1986d). These clefts are probably the result of abnormal stresses induced in physeal cartilage by aberrant limb angulation and altered functional torque. The histological appearence of physeal clefts in the present study was similar to the earlier reports in the fowl. In the present study some regions of cleft formation showed occlusion of physeal vasculature, chondrocyte death and matrix necrosis. In some lesions however minimal PEV and physeal

disruption was apparent. In these cases the presence of a cleft was only noted by the occurrence of a fine sheet of blue dye in the physis. The clefts were site specific in each bone extremity suggesting a localized susceptibility. In the present study the predilection site for physeal clefts was in the femur and proximal tibiotarsus of the overloaded limb. These clefts were probably the result of increased functional loadbearing. In the contralateral limb there were clefts in the physes of distal tibiotarsi and proximal tarsometatarsi. These clefts probably developed in response to altered stresses, induced by abnormal intertarsal angulation of that limb.

One lesion in the proximal tibiotarsus of a load bearing limb in the present study bore similarity to Osgood Schlatters disease. The condition in man is a partial avulsion of the tibial tuberosity (Osgood, 1903; La Zerte and Rudd, 1958), with avulsion involving only the anterior portion of the physis. The condition is considered to be due to excessive functional stress at the site of attachment of the patellar ligament (Ehrenborg and Engfeldt, 1961; Ehrenborg et al, 1961). The cellular changes associated with secondary ossification may predispose the tibial tuberosity to failure (Ogden et al, 1980). Osteochondrosis of the tibial tuberosity has been considered to be involved in the aetiology of the condition (Pappas, 1967). In the present study the lesion was in the cranial physis/epiphysis of the proximal tibiotarsus, between the EOC and the metaphysis. There was disruption of the vasculature and a cleft in the physeal cartilage associated with disruption of local endochondral ossification. At the same site

in S line and a broiler fowls, from experiments 3 and 4, occlusion of PEVs and EVCs was seen to occurr.

There are no apparent references in the literature to an accessory ossification centre in the lateral aspect of the cartilaginous epiphysis of the proximal tarsometatarsus. No EOC occurred at this site in the S line birds of experiment 3. In the ad libitum fed broiler fowls of experiment 4 there was an accessory EOC in the proximal tarsometatarsus of two specimens, but they were not surrounded by traumatic clefts. The accessory EOC may represent a relic of a tarsal centre, which is only occasionally evident.

In man calcified masses sometimes occur in the para articular tissues of the knee. Such masses may appear to be continuous with the bone proper but they are always separated by a radiolucent line. Histological investigations demonstrated that calcification had occurred in the aponeurotic membrane that is adjacent to the medial collateral ligament (Nachlov and Olpp, 1945). In the present study the accessory EOC was an integral part of the cartilaginous epiphysis and not comparable with para articular calcifications recorded in man.

The accessory EOC is deep to the site of attachment of the lateral collateral ligament and as such would be subject to traction forces. Duff (1986e) reported radiodense foci in the distal tibiotarsus at the point of attachment of the lateral collateral ligament. The radiodensities were due to woven bone deposition and were associated with intercondylar ligament rupture in the growing broiler. A traumatic aetiology was suggested,

causing haemorrhage and ossification at the site of lateral collateral ligament attachment to the distal tibiotarsus (Duff, 1986e). In man endochondral ossification has been found to occur in response to trauma at the tendinous insertions of muscles (Hirsch and Morgan, 1939).

In the present study a similar traumatic aetiology is suspected in the formation of the accessory EOC. The accessory EOCs in the two broilers reported in experiment 4 may have enlarged so as to obscure the earlier pathology suggestive of trauma. The traumatic clefts in the unilateral weight bearing fowl of the current experiment however confirm this suggested pathogenesis. The traumatic separation of the medial or lateral collateral ligament from the tarsometatarsus has been recognised a cause of spontaneous injury of the hock joint in the fowl (Craig, 1965). This suggests that the intertarsal joint in the fowl may frequently be subjected to trauma.

In the present study occlusion of the EVCs in the craniomedial femoral head was frequently seen. There was revascularization of the avascular regions in the femoral head by EVCs dividing and branching as they descended from the the capital femoral ligament. The EVCs then formed PEVs which re-established cell hypertrophy and some of them extended into the thickened physeal cartilage occupying the metaphysis. Similarily in a report by Duff (1984a) there was thickening of the physeal cartilage in some examples of PEV/EVC occlusion. EVCs from the capital femoral ligament have been reported in the fowl as forming an EOC as part of the repair process in dyschondroplasia of the

femoral head (Duff, 1984b). In the present study other examples have been described where revascularization of the cartilaginous epiphysis and physis occurred. In the bone extremities the commonest EVCs to be absent were those derived from the ICRVs.

Lowther et al (1974) suggested that the avascularity of dyschondroplastic lesions resulted in impairment of the degradation and biosynthesis of proteoglycans in the matrix of the cartilage. Proteoglycans play an important role in the mineralization of cartilage matrix (Bernard et al, 1977). Any defect in the cartilage proteoglycans may prevent mineralization of the matrix. It seems reasonable to suggest that the faster the production of physeal cartilage and the more rapid the turnover of matrix the greater the likelihood of abnormal proteoglycans formation. Ultrastructural studies in the growing fowl have demonstrated that calcified matrix forms a template for osteoblast aggregation (Howlett, 1980). Hypertrophied chondrocytes form spaces which saccular protrusions from the MVs advance into. It seems reasonable to postulate that the formation of a narrow band of matrix which is not readily calcified would provide an effective barrier to MV penetration. More rapid growth rates would result in a thicker barrier of abnormal cartilage. In the present study the dyschondroplastic lesions were all similar, demonstrating a build up of prehypertrophied chondrocytes and a failure of matrix calcification. Vessels failed to penetrate the abnormal mass of cartilage, and the MVs were blunt ending and tortuous. The retained cartilage underwent regressive changes, presumably because it was avascular. Poulos (1980), in studies on

dyschondroplasia in turkeys, reported a failure of chondrocyte differentiation from transitional to hypertrophied cells and matrix calcification did not occur.

Earlier experiments in the present study demonstrated that normal calcification of physeal cartilage requires the presence of MVs. A band of abnormal cartilage, preventing MV penetration, would similarly block the calcification of normal matrix subsequently produced. This would result in an area of thickened physeal cartilage at the base of which there was failure of MV penetration.

The repair and recovery from dyschondroplastic lesions appeared to involve two processes. These were:

1) The re-establishment of endochondral ossification.

2) The removal of abnormal physeal cartilage.

The abnormal physeal cartilage that occupied the metaphyses was slowly eroded by enlarged MVs from the base of the lesion. A similar MV morphology was described in hypocalcaemic rickets, where there was a failure of MVs to penetrate thickened physeal cartilage (Lacey and Huffer, 1982). Ultrastructural studies in the chick embryo, have provided evidence for a suggested mechanism of resorbtion in the cartilage models of long bones by MV-type vessels (Silvestrini et al, 1979). There was no calcification of matrix prior to MV invasion. The cartilage proteoglycans were digested by enzymes, which were produced by cells in the MVs. These cells subsequently matured into macrophages to remove the rest of the matrix (Silvestrini et al, 1979). In the present study the large mushrooming MVs slowly eroded the base of the

retained cartilage bore a lot of similarities to the description of cartilage resorbtion in the chick embryo. The impression in the present study was that the layer of cartilage at the base of the dyschondroplastic lesions was innately resistant to MV invasion. There was minimal calcification of the matrix and little or no hypertrophy of chondrocytes.

In the majority of dyschondroplastic physes attempts were being made to re-establish endochondral ossification in the retained cartilage above this barrier. Three vascular sources penetrated the physeal cartilage to re-establish chondrocyte hypertrophy and matrix calcification:

1) Elongated PEVs.
2) Perichondrial ring derived MVs.
3) MVs that had circumvented the periphery of the retained cartilage.

The ability of vessels to penetrate this cartilage re-establishing its maturation testifies to its normality. When vitamin D was administered to fowls with vitamin D deficient hypocalcaemic rickets, the PEVs reduced to a normal length and induced calcification of that matrix (Huffer and Lacey, 1982). In the present study it was also demonstrated that in a disease state PEVs can calcify physeal matrix. In the pig PEVs have been described as functioning in the repair of physeal osteochondrosis, and they can re-establish centres of ossification in abnormally thickened physeal cartilage (Kincaid and Lidvall, 1982). The

connective tissue of the PEVs is considered to be a source of osteogenic cells (Lutfi, 1970b; Hunt et al, 1979). A similar function could be ascribed to MV and perichondrial ring derived vessels in the present study. The MVs, and perichondrial derived MVs would also be a source of osteogenic cells. The introduction of osteogenic cells into physeal cartilage at sites of chondrocyte hypertrophy and matrix calcification would enable the physis to return to normal function above the dyschondroplastic lesion.

CONCLUSIONS

Methods have been developed which enabled microangiographic and histological studies to be performed on the same bone extremity. These methods could then be applied to bone extremities of the pelvic appendicular skeleton in a large number of fowls. This information in conjunction with gross morphological examination of long bones provided a picture of normal and abnormal bone growth.

Study of "normal" skeletons in the fowl established many similarities with vascular patterns in other species, and provided insight into the development of vascularity in bone extremities. In addition, the evolution of epiphyseal ossification centres has been clarified. In broiler fowls, patterns of vascular canals in the epiphyseal hyaline cartilage were very similar to those observed in S line fowls. The basic pattern of cartilage canals therefore is not affected by genotype. Physeal vasculature, however, was frequently disrupted in rapidly growing broilers, and was seen in conjunction with a far greater variation in gross morphology of individual long bones. In the "normal" S line fowls small areas of disrupted endochondral ossification were present in some bone extremities. These lesions were much smaller than the comparable but gross pathological changes in broiler fowls. Observations suggested that pathological lesions develop in broiler fowls which cannot undergo repair sufficiently rapidly.

An inability of the vasculature to meet metabolic requirements apeared to contribute to the development of physeal lesions. Reduced growth rate, in broiler fowls, markedly improved

vascular morphology in bone extremities, and considerably fewer lesions developed. Ad libitum fed broilers showed lassitude, but in response to exercise improved physeal vasculature and fewer physeal lesions developed.

Asymmetry of torsion between long bones of the right and left limbs was reduced by feed restriction and also by exercise. It was concluded that localized disruption of endochondral ossification contributed to limb asymmetry and to the greater range of torsion in individual long bones. It became apparent that the development of orthopaedic disease is not a direct consequence of genotype. Bone extremities which were growing at faster rates, developed lesions, which were attributed to inadequate vascularity. Ad libitum feeding of broiler fowls induced behavioural characteristics which were detrimental to the maintainance of vascular perfusion in the growing bone extremities. In broilers the imposition of environmental conditions similar to those enjoyed by birds of "normal" genotype caused the skeleton to follow a more conventional pattern of development.

Studies on the formation and repair of dyschondroplastic lesions gave credence to the hypothesis that a thin layer of abnormal cartilage blocked MV penetration, and gave rise to extensive "plugs" of retained physeal cartilage. Avascularity of retained cartilage would result in degenerative changes and delay in revascularisation.

Rapidly growing bone extremities in broiler fowls were highly susceptible to interruptions in blood supply. Such intrerruptions

would cause a layer of abnormal cartilage to be formed in the physis. The growth rate of broiler fowl physes was up to 50% greater than S line fowl. Abnormal cartilage formed would be 50% thicker in broiler fowls, and such a lesion would take 50% longer to be repaired. The growth rate of broilers exceeds the rate at which some lesions can be repaired and this leads to gross skeletal defects. The propensity of ad libitum fed broiler fowls to severely disrupted endochondral ossification, results in a fowl destined to suffer orthopaedic disease, especially when reared under modern husbandry conditions.

REFERENCES

ALBREKTSSON, T. (1981). Microangiography of bone. Clinic. Orthop. Rel. Res. 159, 285-293.

AGRAWAL, P., ATRE, P.R. & KULKARNI, D.S. 1984. The role of cartilage canals in the ossification of the talus. Acta. Anat. 119, 238-240.

AMPRINO, R. 1985. The influence of stress and strain in the early development of shaft bones. Anat. Embryol. 172, 49-60.

ARDAN, G.M. 1953. In: "Modern Trend in Diagnostic Radiology" ed. J.W. McLaren, London, Butterworth & Co.

ARKIN, A.M. & KATZ, J.F. 1959. The effects of pressure on epiphyseal growth. J. Bone Jt. Surg. 38A, 1056-1076.

AUER, J.A., MARTINS, R.J. & MORRIS, E.L. 1982. Angular limb deformities in foals. Compend. Cont. Educ. for the practicing vet, 4, 330-339.

AUER, J.A., MARTINS, R.J. & MORRIS, E.L. 1983. Angular limb deformities in foals. Compend. Cont. Educ. for the practicing vet, 5, 27-35.

AXER, A., KARPLUS, H., RZETELNY, V. & STYR, J. 1972. Torsional deformation of the femur in rabbits. Israel J. Med. Sci. 8, 43-52.

BALTADJIGEV, G. 1981. Structural peculiarities of the blood vessels supplying the metaphyseal growth cartilage of the tibia in newborns. Folia Med. XXIII 49-53.

BARNETT, C.H. & LEWIS, O.J. 1958. The evolution of some traction epiphyses in birds and mammals. J. Anat. 92, 593-601.

BARNETT, C.H. 1954. A comparison of the human knee and avian ankle. J. Anat. 88, 59-70.

BASSETT, C.A.L. 1962. Current concepts of bone formation. J. Bone J. Surg. 44A 1217-1244.

BASSETT, F.H., WILSON, J.W., ALLEN, B.L. and AZUMA, H. 1967. Normal vascular anatomy of the head of the femur in puppies with emphasis on the inferior retinacular vessels. J. Bone Jt. Surg. 51A, 1139-1153.

BAUMEL, J.J. 1979. Osteologica In: "Nomina Anatomica Avium". ed. Baumel, J.J. Academic Press Inc. London.

BEAUMONT, 1967. The intraosseous vascularisation of the ulna of Gallus domesticus. J. Anat. 101, 543-554.

BERGMAN, V. von & SCHEER, J. 1975. Differential diagnosis of motor disturbance in the pullet. M.H. Vet. Met. 31, 576-581.

BENGIN, B. 1980. Blood supply of chondroapophyseal insertion of the tendon. Vet. Arhiv. 50, 307-317.

BERNARD, B., STAGNI, N., COLAUTTI, I., VITTUR, F. & BONUCCI, E. (1977). Glycosaminoglycans and endochondral calcification. Clin. Orthop. & Rel. Res. 126, 285-291.

BHATNAGAR, R.G., CHRISTIAN, T., NAKANO, F.X., AHERNE, J.R. and THOMPSON 1981. Age-related changes and osteochondrosis in swine articular and epiphyseal cartilage. Canad. J. Comp. Med. 45, 188-195.

BISGARD, J.D. & BISGARD, M.E. 1935. Longitudinal growth of long bones. Arch. Surg. 31, 568-578.

BONUCCI, E. & REURINK, J. 1978. The fine structure of decalcified cartilage and bone: A comparison between decalcification procedures performed before and after embedding. Calcified Tissue Res. 25, 179-190.

BRASHEAR, H.R. 1963. Epiphyseal avascular necrosis and its relation to longitudinal bone growth. J. Bone Jt. Surg. 45A, 1423-1438.

BRIGHT, R.W. & ELMORE, S.J. 1968. Physical properties of epiphyseal plate cartilage. Surg. Forum, 19, 463-464.

BRIGHT, R.W., BURSTEIN, A.H. & ELMORE, S.M. 1974. Epiphyseal plate cartilage. J. Bone Jt. Surg. 56A, 688-703.

BRIGHTON, C.T., RAY, R.D., SABLE, L.W. & KUETTNER, K.E. 1969. In vitro epiphyseal plate growth in various oxygen tensions. J. Bone Jt. Surg. 51A, 1383-1396.

BRIGHTON, C.T. 1978. Structure and function of the growth plate. Clin. Orthop. and Rel. Res. 136, 22-32.

BRODIN, H. 1955. Longitudinal bone growth - the nutrition of the epiphyseal cartilage and the local blood supply. Acta Orthop. Scan. XX 34-43.

BROOKES, M. 1958a. Vascular architecture of tubular bone in the rat. Anat. Rec. 132, 25-34.

BROOKES, M. 1958b. The vascularisation of long bones in the human foetus. J. Anat. 92, 261-267.

BROOKES, M. 1963. Cortical vascularisation and growth in foetal tubular bones. J. Anat. 97, 597-609.

BROOKES, M. & HARRISON, R.G. 1957. The vascularisation of the rabbit femur and tibiotarsus. J. Anat. 91, 61-72.

BROOKES, M. & LANDON, D.N. 1964. The juxta-epiphysial vessels in the long bones of foetal rats. J. Bone Jt. Surg. 46, 336-345.

BRUCE, H.M., KON, S.K., WATSON, J.V., COTCHIN, E. & WHITE, E.G. 1946. A radiographic study of the normal development of the hock joint of the fowl. J. Comp. Path. 56, 49-52.

BULLOUGH, P.G. & HEARD, T.W. 1967. Pathological lesions associated with the "Leg weakness" syndrome in pigs. Br. Vet. J. 123, 305-310.

CAMERON, D.A. 1961. Erosion of the epiphysis of the rat tibia by capillaries. J. Bone Jt. Surg. 43B, 590-594.

CERNY, H. 1983. Ultrastructure of blood vessels in the course of endochondral ossification in cattle. Acta. Vet. Brno 52, 15-20.

CHURCH, L.E. & JOHNSON, L.C. 1964. Growth of long bones in the chick. Am. J. Anat. 114, 521-538.

CRAIG, F. 1967. Traumatic hock disorders in poultry Proc. of Western Poultry Disease Conference, Davis, California, p. 11-12.

CRAIG, P.H. & RISER, W.H. (1965). Osteochondritis dissecans in the proximal humerus of the dog. J. Amer. Vet. Radiol. Soc. 6, 40-49.

CRISSMAN, R.S. & LOW, F.N. 1974. A study of fine structural changes in the cartilage to bone transition within the developing chick vertebrae. Amer. J. Anat. 140, 451-470.

CROCK, H.V. 1967. The blood supply of the lower limb bones in man. Churchill Livingston.

CUNNINGHAM, B. 1966. Bilateral femoral epiphyseolysis in a landrace boar. Irish Vet. J. 20, 66-68.

DIFFORD, J. 1974. A simplified method for the preparation of methyl methylacrylate embedding medium for undecalcified bone. Med. Lab. Technol. 31, 79-91.

DIGBY, K.H. 1916. The measurement of diphyseal growth in proximal and distal directions. J. Anat. 50, 187-188.

DUFF, S.R.I. 1979. Induced abnormal bone growth with particular reference to the growth plate. Ph.D thesis, Fac. Vet. Med. University of Glasgow.

DUFF, S.R.I. 1984a. Dyschondroplasia of the caput femoris in skeletally immature broilers. Res. Vet. Sci. 37, 293-302.

DUFF, S.R.I. 1984b. Capital femoral epiphyseal infarction in skeletally immature broilers. Res. vet. Sci. 37, 303-309.

DUFF, S.R.I. 1984c. Consequences of capital femoral dyschondroplasia in young adult and skeletally mature broilers. Res. Vet. Sci. 37, 310-319.

DUFF, S.R.I. 1985a. Dyschondroplasia/Osteochondrosis of the femoral trochanter in the fowl. J. Comp. Path. 95, 364-371.

DUFF, S.R.I. 1985b. Hip instability in young adult broiler fowls. J. Comp. Path. 95, 374-382.

DUFF, S.R.I. 1985c. Avulsion of muscles from the femoral trochanter in the fowl. J. Comp. Path. 96, 385-392.

DUFF, S.R.I. 1986a. Morphological and radiological findings in induced abnormal bone growth in lambs. J. Comp. Path. 96, 3-13.

DUFF, S.R.I. 1986b. Histopathology of growth plate changes in induced abnormal bone growth in lambs. J. Comp. Path. 96, 14-23.

DUFF, S.R.I. 1986c. Vascular changes in induced abnormal bone growth in lambs. J. Comp. Path. 96, 25-33.

DUFF, S.R.I. 1986d. Windswept deformity in poultry. J. Comp. Path. 96, 147-158.

DUFF, S.R.I. 1986e. Rupture of the inter condylar ligament in intertarsal joints of broiler fowls. J. Comp. Path. 96. 159-170.

DUFF, S.R.I. 1986f. Effect of unilateral weight bearing on pelvic limb development in broiler fowls. Morphological and radiological findings. Res. Vet. Sci. (in press).

DUFF, S.R.I. & RANDALL, C.J. 1986. Observation on femoral head abnormalities in broilers. Res. Vet. Sci. (in press).

DUFF, S.R.I. & HOCKING, P.M. 1986. An assessment of orthopaedic disease in adult male broiler breeding fowls. Res. Vet. Sci. (In press).

DUFF, S.R.I. & THORP, B.H. 1985a. Patterns of physiological bone torsion on the pelvic, appendicular skeleton of domestic fowl. Res. Vet. Sci.

DUFF, S.R.I. & THORP, B.H. 1985b. Abnormal angulation/torsion of the pelvic appendicular skeleton in broiler fowl: Morphological and radiological findings. Res. Vet. Sci. 39, 313-319.

DUTHIE, R.B. & HOUGHTON, G.R. 1981. Constitutional aspects of the osteochondroses. Clin. Orthop. & Rel. Res. 158, 19-27.

DUTHIE, I.F. & LANCASTER, M.L. 1964. Polyarthritis and epiphyseolysis of pigs on England. Vet. Rec. 76, 263-273.

EDWARDS, H.M. 1983. Etiology of tibial dyschondroplasia in poultry. Proc. Georgia Nutritional Conference. p. 103-111.

EHRENBORG, G. & ENGFELDT, B. 1961. The insertion of the ligamentum patellae on the tibial tuberosity. Acta Chir. Scand. 121, 491-499.

EHRENBORG, G., ENGFELDT, B. & OLSSON, S.E. 1961. On the aetiology of Osgood-Schlatter lesions. Acta. Chir. Scand. 122, 445-457.

ELLIOT, J.I. & DOIGE, C.E. 1973. Effects of type of confinement on performance and on the occurrence of locomotory disturbances in market pigs. Canad. J. Anim. Sci. 53, 211-217.

EWBANK, R. 1974. The influence of diet on general activity in fattening pigs. Proc. Third Nat. Pig Vet. Soc. Cong. Lyon.

FELL, B.F., JONES, A.S., & BOYNE, R. 1967. Lesions of distal ulnar epiphysis in wild East African pigs. Vet. Rec. 80, 341.

FELL, H.B. 1925. The histogenesis of cartilage and bone in the long bones of the embryonic fowl. J. Morph. 40, 417-459.

FIRTH, E.C. & POULOS, P.W. 1982. Blood vessels in the developing growth plate of the equine distal radius and metacarpus. Res. Vet. Sci. 33, 159-166.

FIRTH, E.C. & POULOS, P.W. 1983. Microangiographic studies of metaphyseal vessels in young foals. Res. Vet. Sci. 34, 231-235.

FITZGERALD, T.C. 1961. Blood supply of the head of the canine femur. Vet. Med. Vol. 56, 1961, 389-394.

FRANCESCHINI, M.P. 1967. On the appearance and evolution of secondary centres of ossification in the tibia of Gallus Gallus (lina). Acta. Anat. 68, 169-188.

FREDEEN, H.T. & SATHER, A.P. 1978. Joint damage in pigs reared under confinement. Canad. J. Anim. Sci. 58, 759-773.

GANZ, R., LUTHI, U., RAHN, B. & PERRIN, S.M. 1981. Intraartikulare Druckerhohung und epiphysare Durchblutungsstorung. Orthopade. 16, 6-8.

GARDNER, E. & GRAY, D.J. 1970. The perinatal development of the human femur. Am. J. Anat. 129, 121-140.

GEGENBAUR, C. 1864. Untersuchungen zur vergleichenden corpus and tarsus Anatomie der Wirbelthiere, erstes het: 1-127. (cited by Neilson 1963).

GEISER, M. & TRUETA, J. 1958. Muscle action, bone rarefaction and bone formation. J. Bone Jt. Surg. 40B, 282-312.

GOEDEGEBAURE, S.A., HANE, H.J., van der VALK, P.C. & van der WAL, P.G. 1980. Osteochondrosis in six breeds of slaughter pigs. Vet. Quart. 2, 28-41.

GOLDING, J.S.R. & McNEIL-SMITH, J.D.G. 1963. Observations on the aetiology of tibia vara. J. Bone Jt. Surg. 45B, 320-325.

GOTHMAN, L. 1960. The normal arterial pattern of the rabbit's tibia. Acta. Clin. Scand. 120, 201-210.

GRAY, D.J. & GARDNER, E. 1969. Perinatal development of the human humerus. Amer. J. Anat. 124, 431-446.

GRONDALEN, T. 1970. Osteochondrosis and arthrosis in pigs. II. Incidence in breeding animals. Acta. Vet. Scand. 15, 26-42.

GRONDALEN, T. & GRONDALEN, J. 1974a. Osteochondrosis and arthrosis in pigs. II. Incidence in breeding animals. Acta. Vet. Scand. 15, 26-42.

GRONDALEN, T. & GRONDALEN, J. 1974b. Osteochondrosis and arthrosis in pigs. IV. Effect of overloading on the distal epiphyseal plate of the ulna. Acta. Vet. Scand. 15, 53-60.

GRONDALEN, T. & VAUGHEN, O. 1974. Osteochondrosis and arthrosis in pigs. V. A comparison of the incidence in three different lines of the Norwegian Landrace breed. Acta. Vet. Scand. 15, 61-79.

HAINES, R.W. & MOHUIDDIN, A. 1962. Epiphyseal growth and union in the pigeon. J. Fac. Med. Baghdad 4, 4.

HALES, S. 1727. Statistical essays, vol. 1. Vegetable Staticks, London. W. Innys & others p. 337.

HALL-CRAGG, E.C.B. 1968. The effect of experimental epiphysiolysis on growth of the rabbit tibia. J. Bone Jt. Surg. 50B, 392-400.

HAMILTON, G.F., TURNER, A.S., FERGUSON, J.G. & PHARR, J.W. 1978. Slipped capital femoral epiphysis in calves. J. Amer. Vet. Med. Ass. 172, 1318-1322.

HANSSON, L.I., MENADER-SELLMAN, K., STENSTRON, A. & THOMGREN, K.G. 1972. Rate of normal longitudinal growth in the rat. Calc. Tiss. Res. 50, 238-251.

HANSEN-LETCH, C. 1979. The vascularisation in the amputation stumps of rabbits. Acta. Orthop. Scan. 50, 389-406.

HARALDSSON, S. 1962. The vascular patterns of a growing and full grown human epiphysis. Acta. Anat. 48, 156-167.

HARRIS, H.A. 1926. The growth of long bones in childhood. Arch. Nat. Med. XXX VIII 785.

HARRIS, H.A. 1929. The vascular supply of bone, with special reference to the epiphyseal cartilage. J. Anat. Lond. 64, 1-4.

HARTY, M. 1953. The blood supply of the femoral head. Brit Med. J. 2, 1236.

HARVEY, W.M. & D.C. van SICKLE, 1971. A preliminary report on the histopathology of canine degenerative joint disease. Morristoun, N.J., Seeing Eye Foundation Inc.

HEDHAMMER, A. & KROOK, L. & WU. 1974. Overnutrition and skeletal disease. Cornell Vet. 64, suppl. 4-5, 9-131.

HESTER, P.Y., ELKIN, R.G. & KLINGENSMITH, P.M. 1983. Effects of high intensity step up and low intensity step down lighting programs on the incidence of leg abnormalities in turkeys. Poul. Sci. 62, 887-896.

HESTER, P.Y., PENY, K., ADAMS, R.L., FURAMOTO, E.J., LARSEN, J.E., KLINGENSMITH, P.M. PIKE, O.A. & STADELMAN, W.J. 1986. Comparison of two lighting regimes and drinker cleaning programs on the performance and incidence of leg abnormalities in turkey males. Brit. Poult. Sci. 27, 63-73.

HIBBEN, J.H. 1937. The preservation of biological specimen by means of transparent plastic. Science. 86, 247-248.

HILL, M.A. 1984. Observation on the skeleton of young commercial pigs with reference to dyschondroplasia including that called osteochondrosis. Vet. Sci. Dissertation Abstracts International 45, 2464B.

HILL, M.A., HILLEY, H.D., FEENEY, D.A., RUTH, G.R. & HANSGREN, D.C. 1984. Dyschondroplasias, including osteochondrosis, in boars between 25 and 169 days of age: Radiological changes. Amer. J. Vet. Res. 45, 917-925.

HILL, M.A., RUTH, G.R., BAYENT, J.K., TORRISON, J.L. & LEMEN, A.D. 1985. Angiomicrographic investigation of the vessels associated with physes in young pigs. Res. Vet. Sci. 38, 151-159.

HIRSCH, E.F. & MORGAN, R.H. 1939. Causal significance to the traumatic ossification of the fibro cartilage in tendon insertions. Arch. Surg. 39, 824-837.

HLADIKOVA, J. 1981. Endochondral ossification in the electron microscope image. Folia Morphol. XXIX, 139-141.

HOGG, D.A. 1977. Ph.D thesis, Dept of Anatomy, University of Glasgow.

HOGG, D.A. 1980. A re-investigation of the centres of ossification in the avian skeleton at and after hatching. J. Anat. 130, 725-743.

HOORENS, J., OYAERT, W. & THOONEN, H. 1966. Epiphysiolysis of the femoral head in the pig. Vlaam. Diergeneesk. Tijdsch. 35, 16-20.

HOWLETT, C.R. (1979).. The fine structure of the proximal growth plate and metaphysis of the avian tibia. J. Anat. 128, 377-399.

HOWLETT, C.R. 1980. The fine structure of the proximal growth plate and metaphysis of the avian tibia: endochondral osteogenesis. J. Anat. 130, 745-768.

HUFFER, W.E. & LACEY, D.L. 1982. Studies on the pathogenesis of avian rickets. II. Necrosis of perforating epiphyseal vessels during recovery from rickets in chicks caused by vitamin D_3 deficiency. Amer. J. Path. 109, 302-309.

HULTH, A. & WESTERBORN, O. 1963. Early changes of epiphyseal cartilage following immobilisation. J. Traum. 3, 235-242.

HUNT, C.D., OLLERICH, D.A. & NIELSON, F.H. 1979. Morphology of the perforating cartilage canals in the proximal tibial growth plate of the chick. Anat. Rec. 194, 143-158.

HUNTER, W. 1742. Of the structure and diseases of articular cartilages. Phil. Trans. vol. XII, 514-521. (cited Harris 1929).

ITAKURA, C., ASADA, T., GOTO, M. & YAMAGIWA, S. 1975. Histology of the development process of the femoral diaphysis and its pathological significance in broiler chickens. Jap. J. Vet. Sci. 37, 253-260.

JENSEN, R., PARK, R.D., LAUERMAN, L.H., BRADDY, P.M., HORTON, D.P., FLACK, D.E., COX, M.F., EINERTON, N., MILLAR, G.K. & REHFELD 1981. Osteochondrosis in feedlot cattle. Vet. Path. 18, 529-535.

JOHNSON, K.A. 1981. Retardation of endochondral ossification at the distal ulnar growth plate in dogs. Aust. Vet. J. 57, 474-478.

JULIAN, R.J. & BHATNAGAR, M.K. 1985. Cartilage lesions associated with shaky-leg lameness in turkeys. Avian Dis. 29, 218-232.

JEUSILA, J. & PAATSAMA, S. 1972. Radiological changes in the distal epiphysis, epiphyseal cartilage and metaphysis of the radius and ulna in pigs. Acta. Radiol. Suppl. 319, 121-126.

KADERLY, R.E., ANDERSON, W.D. & ANDERSON, B.G. 1982. Extraosseous vascular supply in the mature dog's coxofemoral joint. Amer. J. Vet. Res. 43, 1208-1214.

KADERLY, R.E., ANDERSON, B.G. & ANDERSON, W.D. 1983. Intracapsular and intraosseous vascular supply in the mature dog's coxofemoral joint. Amer. J. Vet. Res. 44, 1805-1812.

KELLY, J.K. & JANES, J.M. 1968. Microangiographic and histologic studies of vascular anatomy of the femur and tibia distal in femoral and arteriovenus fibula in dogs. Anat. Rec. 162, 255-267.

KELLY, P.J., PETERSON, L.F.A. & JANES, J.M. 1959. A method of using sections of bone prepared for microangiography for subsequent histological study. Proc. Staff Meet. May Clin. 34, 274-283.

KEMBER, N.F. 1960. Cell division in endochondral ossification. J. Bone Jt. Surg. 42B, 824-830.

KEMBER, N.F. & SISSONS, H.A. 1976. Quantitative histology of the human growth plate. J. Bone J. Surg. 8B, 426-435.

KINCAID, S.A. & LIDVALL, E.R. 1982. Communicating cartilage canals of the physis of the distal part of the ulna of growing swine and their potential role in healing of metaphyseal dysplasia of osteochondrosis. Amer. J. Vet. Res. 43, 938-944.

KINCAID, S.A. & LIDVALL, E.R. 1983. Observations on the post-natal morphogenesis of the porcine humeral condyle and the pathogenesis of osteochondrosis. Amer. J. Vet. Res. 44, 2095-2103.

KUGLER, J.A., TOMLINSON, A., WARD, S. & WAGSTAFF, A. 1978. Autoradiography and ultrastructure study on cartilage canals. Proc. of the Anatomical Society of Great Britain and Ireland, Jan. 1978. J. Anat. 126, p. 426.

KUGLER, J.H., TOMLINSON, A., WAGSTAFF, A. & WARD, S.M. 1979. The role of cartilage canals in the formation of secondary centres of ossification. J. Anat. 129, 493-506.

LACEY, D.L. & HUFFER, W.E. 1982. Studies on the pathogenesis of avian rickets. I. Changes in epiphyseal and metaphyseal vessels in hypocalcaemic and hypophosphataemic rickets. Amer. J. Path. 109, 288-301.

LANGER, K. 1876. Uber das gefassystem der rohrenknochen. Denkschriften der kaiserlichen. Akademie der Wissenschaften. 36, 1.

LANGENSKIOLD, A. 1981. Tibia vara: Osteochondrosis deformans tibiae. Clin. Orthop. & Rel. Res. 158, 77-82.

LANDSDOWN, A.B.G. 1970. A study of the normal development of the leg skeleton in the quail. J. Anat. 106, 147-160.

LANYON, L.E. & BAGGOT, D.G. 1977. The functional adaptation of bone. J. Bone Jt. Surg. 59B, 250.

LANYON, L.E. & BOURN, S. 1979. The influence of mechanical formation on the development and remodelling of the tibia. An experimental study in sheep. J. Bone Jt. Surg. 61A, 263-273.

LATIMER, H.B. 1927. Post-natal growth of the chicken skeleton. Am. J. Anat. 40, 1, 1-57.

LAW, E.G., HEISTAD, D.D., MARCUS, M.L. & MICKELSON, M.R. 1982. Effect of hip position on blood flow in the femur in puppies. J. Pediat. Orthop. 2, 133-137.

La ZERTE, G.D. & RUDD, I.H. 1958. Pathogenesis of Osgood-Schlatter's Disease. Amer. J. Path. 34, p. 803-815.

LEACH, R.M. & NESHEIM, M.C. 1965. Nutritional, genetic and morphological studies of an abnormal cartilage formation in young chicks. J. of Nutr. 86, 236-244.

LEVENE, C. 1964. The patterns of cartilage canals. J. Anat. 98, 515-538.

LEWIS, O.J. 1956. The blood supply of developing long bones with special reference to the metaphysis. J. Bone Jt. Surg. 38, 928-933.

LEXER, E. 1904. Untersuchengen uber Knockenarterien mittelst rontgenaufnahmen injizierten knochen. A. Hirshwald Berlin. (cited by Tilling, G. 1958).

LOWTHER, D.A., ROBINSON, H.C., DOLMAN, J.W. & THOMAS, K.W. 1974. Cartilage matrix components in chickens with tibial dyschondroplasia. J. Nutr. 104, 922-929.

LJUNGREN, G. & REILAND, S. 1970. Osteochondrosis in adolescent animals an endocrine disorder? Calc. Tiss. Res. 4, 150-151.

LUND-LARSEN, T.R. & BAKKE, H. 1975. Growth hormone and somatomedin activities in lines of pigs selected for rate of gain and thickness of backfat. Acta. Agric. Scand. 25, 231-234.

LUTFI, A.M. 1970a. Mode of growth, function and fate of cartilage canals. J. Anat. 106, 135-145.

LUTFI, A.M. 1970b. Study of cell multiplication in the cartilagious upper end of the tibia of the domestic fowl by irradiated thymidine autoradiography. Acta. Anat. 76, 454-463.

LUNDSKOG, J., BRANEMARK, P-I. & LINDSTROM, J. 1968. Biomicroscopic evaluation of microangiographic methods. Adv. Microcirc. 1, 152-160. (Kergav, Basel/New York).

MAWHINNY, W.H.B. & ELLIS, H.A. 1983. A technique for plastic embedding of mineralised bone. J. Clin. Path. 36 1197-1199.

MEENS, M.H.C.C. & LITJENS, J.B. 1978. Degeneration of the head of the femur in poultry. Tijd Diergen. 103, 907-909.

MICKELSON, M.R., McCURNIN, D.M., AWBREY, B.J., MAYNARD, J.A. & MARTIN, R.K. 1980. Legg-Calve Perthes disease in dogs. Clin. Orthop. 157, 287-300.

MICKELSON, M.R., PONSETTI, I.V., COOPER, R.R. & MAYNARD, J.A. 1977. The ultrastructure of the growth plate in slipped capital femoral epiphysis. J. Bone Jt. Surg. 59A, 1076-1081.

MORSE, E.S. 1872. On the carpel tarsal bones of birds. Nature. p. 293.

MURBARAK, S.J. & CARROLL, N.C. 1982. Juvenile osteochondritis dissecans of the knee: Etiology Clin. Orthop. 157, 200-211.

NACHLOV, I.W. & OLPP, J.L. 1945. Para-articular calcification in affections of the knee. Surg. Gynecol. Obstet. 81, 206-212.

NAKANO, T., AHERNE, F.X., BRENNAN, J.J. & THOMPSON, J.R. 1984. Effect of growth rate on the incidence of osteochondrosis in growing swine. Canad. J. Anim. Sci. 64, 139-146.

NAVAGIRI, S.S. & DUBEY, P.N. 1975. Epiphysis of the lower end of the chick tibia. J. Anat. Soc. India. 24, 35.

NEWMAN, S.B., BORYSKO, E. & SWERDLOW, M. 1949. Ultra-microtomy by a new method. J. Res. Natl. Bur. Stand. 43, 183-199.

NIELSEN, E.H. 1963. The development of the tarsus in Gallus domesticus. Acta. Vet. Scand. 4, 13-26.

NOSER, G.A., CARRIG, C.B., MERKLEY, D.F. & BRINKER, W.O. 1977. Asynchronous growth of the canine radius and ulna: Effects of cross pinning the radius to the ulna. Amer. J. Vet. Res. 38, 601-610.

OGDEN, J.A. 1981. "Hip" Ch. 10 Development and Vascularity, relationship to chondro-osseous trauma. pp. 139-187.

OGDEN, J.A., HEMPTON, R.F. & SOUTHWIND, W.O. 1974. Development of the tibial tuberosity. J. Bone Jt. Surg. 56A, 7, Proceeding, 1539.

OGDEN, J.A., TROSS, R.B. & MURPHY, M.J. 1980. Fractures of the tibial tuberosity in adolescents. J. Bone Jt. Surg. 62A, 205-215.

OKAWA, C. & TROMBKA, J.I. 1956. The technique of microangiograms of rabbit bone marrow. Amer. J. Clin. Pathol. 26, 758-764.

OLERUD, S., DANCKWARD-LILLIESTROM, G. 1968. Fracture healing in compression osteosynthesis in the dog. J. Bone Jt. Surg. 50B, 844-851.

OLSSON, S.E. 1975. Osteochondritis dissecans in the dog. Proc. Amer. Anim. Hosp. Ass. 42nd Ann. Meeting Cincinnati, Ohio, Proc. Vol. 1 360-370.

OLSSON, S.E. 1976. Osteochondrosis - a growing problem to dog breeders. Gaines Progress, Summer 1976, 1-11.

PARSONS, F.G. 1904. Observation on traction epiphyses. J. Anat. & Physiol. 38, 248-458.

PARSONS, F.G. 1905. On pressure epiphyses. J. Anat. & Physiol. 39, 402-412.

PAUKKONEN, K. SELKAINAHO, K., JURVELIN, J., KIVIRANTA, I. & HELMAN, H.J. 1985. Cells and nuclei of articular cartilage chondrocytes in young rabbits enlarged after non-strenuous physical exercise. J. Anat. 142, 13-20.

PAYTON, C.G. 1932. The growth in length of the long bones in the madder fed pig. J. Anat. 66, 414-425.

PERRIN, W.R. & BOWLAND, J.P. 1977. Effects of enforced exercise on the incidence of leg weakness in growing bones. Canad. J. Anim. Sci. 57, 245-253.

PETERSON, L.F.A., KELLY, P.J. & JANES, J.M. 1957. Ultrastructure of bone: Technic of microangiography as applied to the study of bone. Proc. Staff Meet. Mayo Clinic. 32, 681-686.

POHLYMEYER, von K. 1981. Der articulatio coxae und des proximalen femurabschnittes bei der katze. Zbl Vet. Med. C. Anat. Histol. Embryol. 10, 246-256.

POULOS, P.W. 1978. Tibial dyschondroplasia (osteochondrosis) in the turkey: A morphological investigation. Acta. Rediol. Suppl. 348, 197-227.

POULOS, P.W. 1980. The contributions of osteochondrosis to the leg weakness problem. Turkeys, 28, 45-46.

POULOS, P.W., REILAND, S. & ELWINGER, K. 1978. Skeletal lesions in the broiler, with special reference to dyschondroplasia (osteochondrosis). Acta. Radiol. Suppl. 358, 229-275.

PRASAD, S., HAIRR, W.T. & DALLAS, J.T. 1971. Observations of abnormal cartilage formation associated with leg weakness in commercial broilers. Avian Dis. 16, 457-461.

REILAND, S. 1974. Osteochondrosis related to rapid growth: A common cause of "leg weakness" in breeding pigs. A preliminary report. Sv. Vet. Tidn. 26, 28-33.

REILAND, S. 1978a. Pathology of so called leg weakness. Acta. Radiol. Suppl. 358, 23-44.

REILAND, S. 1978b. The effect of decreased growth rate on frequency and severity of osteochondrosis in pigs. Acta. Radiologica Suppl. 358, 107-122.

REILAND, S., POULOS, P., HANSEN, H.J., LINDGREN, N.O. & OLSSON, S.E. 1977. Tibial dyschondroplasi hos broiler - eff delfenomen; en generaliserand sjukdom - osteochondros. Sv. Vet. Tidn. 29, 123-127.

REILAND, S., STROMBERG, B., OLSSON, S.E., DREIMARIS, I. & OLSSON, I. 1978a. Osteochondrosis in growing bulls. Pathology, frequency and severity on different feedings. Acta. Radiol. Suppl. 358, 179-196.

REILAND, S., OLSSON, S.E., POULOS, P.W. JR & ELWINGER, K. 1978b. Normal and pathologic skeletal development in broiler and leghorn chickens. Acta. Radiologica Suppl. 358, p. 277-298.

REIDY, J.S., LINGLEY, J.R., GALL, E.A. & BARR, J.S. 1947. The effect of roentgen irradiation on epiphyseal growth. J. Bone Jt. Surg. 29, 853-873.

REINHOLD, H.S., HOPEWELL, J.W. & RIJSOORT, A.V. 1983. A revision of the Spalteholz methods for visualizing blood vessels. Int. J. Microcirc. Clin. Exp. 2, 47-52.

REJNO, S. & STROMBERG, B. 1978. Osteochondrosis in the horse II. Pathology. Acta. Radiol. Suppl. 358, 153-178.

RHINELANDER, F.W. & BARAGRY, R.A. 1962. Microangiography in bone healing. J. Bone Jt. Surg. 44A, 7, 1273-1298.

RHINELANDER, F.W. (1968). The normal microcirculation of diaphyseal cortex and its response to fracture. J. Bone Jt. Surg. 50A, 784-800.

RHINELANDER, F.W., PHILLIP, R.S., STEEL, W.M. & BEER, J.C. 1968. Microangiography in bone healing. J. Bone Jt. Surg. 50A, 643-662.

RHINELANDER, F.W,. STEWART, C.L. & WILSON, J.W. 1979. In: "Skeletal research, an experimental approach". Ed. D.J. Simmons and A.S. Kunin, New York Academic Press. 367-395.

RIDDELL, C. 1975a. Studies on the pathogenesis of tibial dyschondroplasia in chicks. I. Production of a similar defect by surgical interference. Avian Dis. 19, 483-489.

RIDDELL, C. 1975b. Studies on the pathogenesis of tibial dyschondroplasia in chickens. II Growth rate of long bones. Avian Dis. 19, 490-505.

RIDDELL, C. 1975c. Studies of the pathogenesis of tibial dyschondroplasia in chickens. III. Effect of body weight. Avian Dis. 19, 497-505.

RIDDELL, C. 1975d. The development of tibial dyschondroplasia in broiler chickens. Avian Dis. 19, 443-462.

RIDDELL, C. 1981. Skeletal deformities in poultry. In: "Advances in Veterinary Science and Comparative Medicine". Ed. Cornelius & Simpson, Academic Press, vol. 25, p. 277-310.

RIDDELL, C., KING, M.W., GUNASEKERA, K.R. 1983. Pathology of the skeleton and tendons of broiler chicks reared to roaster weight. II. Normal chicken. Avian. Dis. 27, 980-991.

RIETZ, K-A. 1968. Polymer osteosynthesis III. Segmental rejection of femur and fixation of endoprosthesis. Acta. Chir. Scand. Suppl. 388.

RISER, W.A. & SHIRER, J.F. 1965. Normal and abnormal growth of the distal foreleg in large and giant dogs. J. Amer. Vet. Rad. Soc. 6, 1950-65.

ROCHE, A.F. 1965. The sites of elongation of human metacarpels and metatarsals. Acta. Anat. 61, 193-202.

RODENHOFF, G. & DAMMRICH, K. 1971. Uber die beeinflussung des skeletts der masthahnchen durch haltung und auslanf in freien. Zbl. Vet. Med. A 18, 297-309.

RODENHOFF, G. & DAMMRICH, K. 1973. Untersuchungen zur beeinflussung der rohrenknockenstruktur durch verschiedene haltungssysteme bei masthahnchen. Berl. u Munch. Tierarztl. Wschr. 241-244.

RODGERS, W.M. & GLADSTONE, H. 1950. Vascular foramen and arterial supply to the distal end of the femur. J. Bone Jt. Surg. 32A, 367-874.

ROMANOFF, A.L. 1960. In: "The avian embryo". Structural and functional development". Ed. Romanoff, A.L. McMillan, New York. 1011-1016.

ROTHWELL, B., BURNS, R.B. & RALPH, J.A. 1973. Multipurpose perfusion apparatus. Lab. Practice, 22, 635.

RUBIN, P. 1964. Microangiography facts and artefacts. Radiological clinics of N. America. 2, 499-513.

SAAF, J.E. 1941. The effect of function on the articular cartilage of young pigs. Acta. Soc. Med. Upsaliensis. 46, 349.

SAAF, J.E. 1950. Effects of exercise on adult articular cartilage. Acta. Orthop. Scand. VII.

SAAF, J.E. (1950).

SALENTIJN, C.M. 1974. Studies of tone bone growth. Acta. Anat. 90, 145-160.

SATHER, A.P. & FREDEEN, H.T. 1982. The effect of confinement housing upon the incidence of leg weakness in swine. Canad. J. Anim. Sci. 62, 1119-1128.

SCAPINELLI, R. 1968. Studies on the vasculature of the human knee joint. Acta. Anat. 70, 305-331.

SCHOENECKER, P.L., BITZ, D.M. & WHITESIDE, L.A. 1978. The acute effect of position of immobilization on capital femoral epiphyseal blood flow. J. Bone Jt. Surg. 60A, 899-904.

SEVITT, S. 1964. A vascular necrosis and revascularization of the femoral head after intracapsular fractures. J. Bone Jt. Surg. 46B, 270-296.

SEVITT, S. & THOMPSON, G. 1965. The distribution and anastomoses of arteries supplying the head and neck of the femur. J. Bone Jt. Surg. 47B, 560-573.

SIEBEL, von S., DAMMRICH, K. & ANDREAE, U. 1973. Uberbelastungsschaden an der distalen epiphysenfugenscheibe des radius bei mastbullen ans verschiedenen aufstallungsformen. Berl. Munch. Tierarztl. Wschr, 86, 441-460.

SILLER, W.G. 1970. Tibial dyschondroplasia in the fowl. J. Pathol. 101, 39-46.

SILLER, W.G. & DUFF, R.H. 1970. Cartilage abnormality in broiler fowls. Vet. Rec. 86, 757.

SILVESTINI, G., RICORDI, M.E & BONUCCI, E. 1979. Resorbtion of uncalcified cartilage in the diaphysis of the chick embryo tibia. Cell & Tissue Res. 196, 221-235.

SIMONS, P.C.M. & HAYE, U. 1985. Intermittent lighting has a positive effect on twisted legs. Poultry - Misset, March.

SISSONS, H.A. 1953. Experimental determination of rate of longitudinal bone growth. J. Anat. 87, 228-236.

SISSONS, H.A. 1971. The growth of bone. In: "The biochemistry and physiology of bone". ed. G.H. Bourne, vol. 3, Academic Press, New York. 145-180.

SMITH, J.W. 1962a. The relationship of epiphyseal plates in stress in some bones of the lower limb. J. Anat. 96, 58-78.

SMITH, J.W. 1962b. The structure and stress relation of fibrous epiphyseal plates. J. Anat. 96, 2, 209-285.

SMUTTS, M.M.S. 1978. The intraosseous blood vessels of the cervical vertibrae of the ox. Proc. Anat. Soc. of S. Africa. J. Anat. 126, 664-665.

SOKOLOFF, L. 1969. In: "The biology of degenerative joint disease" The University of Chicago Press. Chicago.

SPATEHOLZ, (1914). Ueber durchsichtigmachen von menschlichen und tieischen praparaten zweite auflage. Leipzig, S. Hirzel. Cited by Olerud & Dankwardt Lilliestrom (1968).

SPIRA, E. & FARIN, I. 1967. The vascular supply to the epiphyseal plate under normal and pathological conditions. Acta. Orthop. Scand. 38, 1-22.

SPIRA, E., FARIN, I., & KARPLUS, H. 1963. The capillary conducting channels in the distal epiphyses of radius and ulna of the rabbit. J. Anat. 97, 255-258.

STEINKE, 1971. Osteodystrophy - Nutritional and related factors. Proc. Symp. Leg Weakness in turkey, Iowa State Univ.

STOCKWELL, R.A. 1971. The ultrastructure of cartilage canals and the surrounding cartilage in the sheep fetus. J. Anat. 109, 397-410.

STROMBERG, B & REJNO, S. 1978. Osteochondrosis in the horse. I. A clinical and radiological investigation of osteochondrosis dissecans of the knee and hock joint. Acta. Radiol. Suppl. 358, 139-152.

SUORANTA, H. & KORMANO, M. 1974. Fixation induced changes in the microangiographic picture of the blood vessels. Invest. Radiol. 5, 408-41.

TEARIE, J.A., HINTZ, H.F. & KROOK. L. 1980. Rapid growth and skeletal disease in dogs. Proceedings of the Cornell Nutritional Conference. Cornell University Ithaca, New York, U.S.A. 126-130.

TILLING, G. 1958. The vascular anatomy of long bones. Acta. Radiologica Suppl. 161, p. 6-15.

TRUETA, J. 1957. The normal vascular anatomy of the femoral head during growth. J. Bone Jt. Surg. 39B, 358-393.

TRUETA, J. 1963. The role of the vessels in osteogenesis. J. Bone Jt. Surg. 45B, 402-418.

TRUETA, J. & BUHR, A.J. 1963. The vascular contribution in osteogenesis. J. Bone Jt. Surg. 45B, 572-581.

TRUETA, J. & HARRISON, M.H.M. 1953. The normal vascular anatomy of the femoral head in adult man. J. Bone Jt. Surg. 35B, 442-461.

TRUETA, J. & LITTLE, K. 1960. The vascular contribution to osteogenesis. II Studies with the electron microscope. J. Bone Jt. Surg. 42B, 367-376.

TRUETA, J. & MORGAN, J.D. 1960. The vascular contribution to osteogenesis. Studies by the injection method. J. Bone Jt. Surg. 41, 97-109.

TRUETA, J. & TRIAS, A. 1961. The vascular contribution to osteogenesis. IV The effect of pressure upon the epiphyseal cartilage of the rabbit. J. Bone Jt. Surg. 43B, 800-814.

UHTHOFF, H.K., FINNEGAN, N. & HIDIROGLOU, M. 1982. Epiphysiolysis, a possible cause of limb deformities in lambs. Ann. Rech. Vet. 13, 237-244.

VELDE, J.,T.E., BURKHARDT, R., KLEIVEDE, K., LEENHEES-BINNEDIJK, & SOMMERFIELD, W. 1977. Methyl methylate as an embedding media in histopathology. Histopathology 1, 319-330.

VISCO, D.M. & KINCAID, S.D. 1983. Observations on the cartilage canals of the distal humerus of growing pigs. Abs. Annual Meeting of the conference of research workers in animal disease. Chicago, vol. 64, 10.

WALKER, T., FELL, B.F., JONES, A.S., BOYNE, R. & ELLIOT, M. 1966. Observations on "Leg Weakness" in pigs. Vet. Rec. 79, 472-479.

WALSER, M.M., CHERMS, F.L. & DZIUK, H.E. 1982. Osseous development and tibial dyschondroplasia in five lines of turkeys. Avian Dis. 26, 265-271.

WANG, Y.C. 1975. Intrachondral microcirculation. Chinese Med. J. 1, 449-460.

WENK, C. & van ES, A.J.H. 1977. Energy metabolism of growing chickens as related to their physical activity. Proceedings of the 7th Symposium EAAP - Energy Metabolism of farm animals, 189-192.

WERTHEIMER, L.G. & LOPEZ, S.F. 1971. Arterial supply of the femoral head. J. Bone Jt. Surg. 53A, 545-555.

WILSMAN, N.J. & van SICKLE, D.C. 1970. The relationship of cartilage canals to the initial osteogenesis of secondary centres of ossification. Anat. Rec. 168, 381-392.

WILSMAN, N.J. & van SICKLE, D.C. 1972. Cartilage canals, their morphology and distribution. Anat. Rec. 173, 79-94.

WILSON, J.L., WEAVER, W.D., BEANE, W.L. & CHERRY, J.A. 1984. Effects of light and feeding space on leg abnormalities in broilers. Poultry Sci. 63, 565-567.

WISE, D.R. & JENNINGS, A.R. 1973. The development and morphology of the growth plates of two long bones of the turkey. Res. Vet. Sci. 14, 161-166.

WOLBACH, S.B. & HEGSTED, D.M. 1952. Endochondral bone growth in the chick. Arch. Pathol. 54, 1-12.

WOLCOTT, W.E. 1943. The evolution of the circulation in the developing femoral head and neck. Surg., Gynec. & Obst. 77, 61-68.

YABSLEY, R.H. & HARRIS, W.P. 1963. The effect of rickets and of procedures known to stimulate growth on the blood supply to the epiphyseal plate. J. Bone Jt. Surg. 47A, 561-566.

YAMASKI, K. & INUI, S. 1985. Lesions of articular, sternal and growth plate cartilage in rats. Vet. Pathol. 22, 46-50.

KEY TO APPENDICES

Intertarsal angulations

N = No varus or valgus angulation

sl = slight valgus angulation

+1 = 5 degrees valgus angulation

+2 = 10 degrees valgus angulation

var = slight - 5 degrees varus angulation

Torsion

greater than 0 is external

less than 0 is internal

Length is in mm

Processed

P = processed into Polymaster resin blocks

- = stored in buffered neutral formalin

Appendix 1

"Ad Libitum" S line

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion Left			Torsion Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
48	0	-	44	sl	sl	-	-	-	-	-	-	24	32	24	P
50	0	-	42	sl	sl	-	-	-	-	-	-	23	31	23	P
51	0	-	40	+1	N	-	-	-	-	-	-	23	31	23	P
52	0	-	43	sl	+1	-	-	-	-	-	-	24	33	23	-
53	0	-	40	N	N	-	-	-	-	-	-	23	32	-	P
54	0	-	41	sl	+1	-	-	-	-	-	-	25	34	-	-
55	0	-	41	Var	+1	-	-	-	-	-	-	23	33	23	-
56	0	-	39	N	sl	-	-	-	-	-	-	23	30	23	P
93	2	M	35	N	N	5	2	-4	2	3	-9	24	34	24	-
92	2	F	41	N	sl	4	5	-5	6	2	-8	26	34	26	-
89	2	M	37	sl	sl	-2	4	-3	2	6	-6	24	33	24	P
90	2	M	44	sl	sl	7	7	-6	7	4	-7	25	34	25	P
87	2	M	42	sl	sl	0	7	-5	5	4	-5	24	33	24	P
88	2	F	45	sl	sl	4	7	-7	5	5	-6	24	34	24	P
94	2	M	36	sl	sl	8	5	-5	8	0	-5	24	32	24	-
91	2	F	41	+1	sl	3	5	-5	5	2	-6	25	32	25	-
99	5	M	41	sl	+1	6	9	-6	8	6	-6	26	34	26	P
97	5	F	59	sl	sl	6	9	-4	4	9	-7	27	36	26	P
96	5	M	55	sl	+1	8	12	-12	9	7	-10	26	36	26	P
102	5	M	55	+1	sl	4	4	-9	9	4	-6	27	36	26	-
103	5	F	39	N	sl	8	8	-6	5	7	-7	26	35	25	-
98	5	F	49	sl	sl	4	8	-8	3	6	-5	27	36	26	P
101	5	F	47	sl	sl	7	8	-8	12	4	-9	26	34	25	P
100	5	M	42	sl	sl	7	12	-6	7	7	-6	26	34	26	-
143	7	F	51	sl	sl	8	10	-	8	9	-8	26	36	26	-
138	7	M	57	sl	sl	2	14	-6	7	6	-7	27	36	26	P
136	7	M	60	sl	sl	7	13	-5	7	10	-6	27	37	28	P
142	7	M	58	+1	sl	9	7	-7	11	6	-8	25	34	24	-
137	7	F	53	+1	sl	9	10	-8	8	9	-10	29	38	28	P
140	7	M	62	sl	sl	10	5	-5	12	7	-9	28	38	27	-
139	7	F	42	sl	sl	6	10	-7	6	6	-6	29	38	28	P
141	7	F	54	+1	+1	7	7	-6	7	5	-8	27	36	26	-
158	9	F	55	sl	sl	10	9	-13	12	7	-9	26	36	26	-
156	9	F	69	sl	sl	9	6	-9	15	6	-10	28	37	27	P
159	9	F	72	+1	sl	12	6	-7	6	5	-8	27	37	26	-
155	9	F	57	N	sl	15	6	-7	11	4	-7	27	38	27	P
157	9	M	60	sl	sl	6	8	-8	8	3	-8	27	37	27	P
160	9	M	56	+1	sl	8	8	-9	6	8	-9	29	39	28	-
161	9	M	56	sl	sl	6	9	-6	7	12	-8	27	35	27	-
154	9	M	50	+1	sl	6	8	-10	9	12	-8	27	35	26	P

Appendix 1 (contd)

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion degrees Left			Torsion degrees Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
190	14	M	76	sl	sl	13	9	-6	10	9	-12	36	46	35	-
189	14	M	106	sl	+1	18	11	-6	15	8	-9	32	42	31	P
193	14	F	106	+1	+1	14	12	-9	12	7	-9	35	45	33	-
191	14	M	95	sl	+1	12	11	-7	10	2	-9	34	43	32	-
188	14	F	120	+1	+1	17	2	-6	13	4	-9	30	39	29	P
186	14	M	127	sl	+1	14	10	-6	10	12	-8	33	43	32	P
187	14	F	80	sl	+1	14	10	-5	12	7	-9	30	40	29	P
192	14	F	77	+1	+1	-	-	-	-	-	-	33	44	32	-
215	21	F	195	sl	sl	15	15	-8	8	11	-13	39	51	37	P
216	21	F	150	sl	sl	11	8	-8	9	13	-8	42	55	41	-
220	21	F	156	sl	sl	10	12	-9	9	13	-12	39	51	37	-
217	21	M	123	+1	+1	18	8	-7	13	13	-8	40	52	38	-
219	21	M	104	sl	+1	14	14	-8	7	15	-11	34	46	33	-
214	21	M	139	sl	+1	16	6	-9	16	3	-10	40	52	37	P
213	21	M	198	+1	+1	26	3	-7	18	4	-9	44	59	42	P
212	21	F	148	+1	+1	16	6	-8	11	7	-10	39	52	37	P
218	21	M	208	sl	+1	17	15	7	16	19	-12	44	58	42	-
367	28	M	143	sl	sl	12	8	-7	7	13	-11	42	54	39	-
361	28	M	303	sl	sl	18	6	-12	15	5	-15	50	64	46	P
362	28	F	227	+1	+1	16	10	-16	10	12	-12	44	57	42	P
365	28	M	330	+1	+1	20	2	-8	16	12	-12	47	62	47	-
364	28	F	273	+1	+1	10	12	-9	11	12	-14	49	65	43	P
363	28	M	293	+1	+1	17	8	-12	15	16	-12	52	69	51	P
368	28	F	270	+1	+1	12	8	-7	7	13	-11	49	62	47	-
366	28	F	274	+1	+1	10	8	-8	12	8	-9	49	65	47	-
443	42	M	580	+1	+1	18	8	-9	8	14	-13	58	77	57	-
438	42	F	455	+1	+1	19	10	-13	14	8	-11	58	82	58	P
435	42	M	420	+1	+1	15	10	-12	12	14	-12	57	80	57	P
421	42	F	331	+1	+1	10	8	-12	5	3	-8	57	80	57	-
440	42	M	486	+1	+1	16	4	-8	15	6	-12	54	73	50	-
436	42	F	397	sl	sl	14	11	-10	10	4	-13	59	80	57	P
442	42	F	317	+1	+1	16	5	-8	13	10	-10	57	72	54	-
437	42	M	468	+1	+1	22	12	-10	14	8	-15	53	68	50	P

Appendix 1 (contd)

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion degrees Left			Torsion degrees Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
575	70	M	940	+1	+1	12	7	-10	10	4	-17	81	111	79	-
574	70	M	997	+1	+1	10	10	-6	6	8	-12	98	124	88	P
576	70	M	995	+1	+1	14	6	-8	11	8	-15	77	109	77	-
573	70	F	881	s1	+1	15	5	-10	10	7	-13	78	107	80	P
577	70	F	750	+1	+1	12	6	-10	12	4	-4	81	112	82	-
572	70	F	675	s1	+1	14	8	-5	15	8	-7	73	96	71	P
571	70	F	868	+1	+1	9	13	-8	8	16	-12	75	102	74	P
578	70	M	968	+1	+1	12	10	-7	8	5	-9	80	112	85	-
704	105	F	1380	s1	+1	10	2	-9	5	0	-7	88	125	87	-
706	105	M	1350	s1	+1	11	7	-13	6	4	-16	94	138	97	-
703	105	M	1520	s1	+1	10	6	-10	10	4	-13	97	138	100	P
701	105	M	1530	s1	+1	8	7	-10	5	5	-10	98	142	97	P
707	105	M	1520	s1	+1	9	6	-10	11	-4	-13	100	142	105	-
700	105	F	1160	+1	+1	16	5	-12	9	7	-5	90	130	92	P
702	105	F	1190	+1	+1	9	8	-8	11	5	-9	90	129	90	P
705	105	F	1240	+1	+1	10	8	-9	7	4	-11	89	113	88	-
1064	140	M	1950	s1	+1	9	4	-7	5	2	-9	102	149	105	P
1068	140	M	1860	s1	+1	4	2	-9	3	2	-11	100	143	100	-
1066	140	M	1705	s1	+1	8	6	-6	8	10	-9	100	142	98	P
1067	140	M	1820	s1	+1	4	7	-7	0	2	-9	102	150	103	-
1069	140	F	1505	s1	s1	4	1	-6	3	1	-8	90	130	88	P
1070	140	F	1360	s1	s1	7	2	-6	6	2	-7	87	123	83	-
1065	140	F	1610	s1	s1	7	9	-11	5	4	-9	87	125	87	P
1071	140	F	1367	s1	+1	3	5	-7	2	3	-6	92	127	89	-

Appendix 2

"Ad libitum" Broilers

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion degrees Left			Torsion degrees Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
387	0	-	52	N	sl	5	6	-8	4	4	-12	26	33	25	-
383	0	-	37	sl	sl	16	10	-5	14	8	-11	22	31	22	P
384	0	-	47	N	N	16	-8	-10	10	-6	-10	26	35	25	P
388	0	-	45	sl	+1	10	6	-7	4	-8	-9	25	36	25	-
385	0	-	44	sl	sl	12	10	-10	10	13	-13	26	36	25	P
389	0	-	41	Var	N	9	8	-10	7	10	-10	25	33	25	-
386	0	-	43	sl	sl	12	7	-11	12	5	-10	23	32	23	P
390	0	-	47	N	+1	6	7	-10	6	10	-11	25	37	25	-
397	2	F	55	Var	sl	12	0	-8	8	7	-15	-	-	-	-
398	2	M	42	sl	sl	7	3	-9	10	4	-10	-	-	-	-
400	2	M	44	sl	sl	15	9	-8	8	5	-8	-	-	-	-
394	2	M	46	+1	+1	12	6	-6	11	11	-7	-	-	-	P
395	2	M	43	sl	N	14	2	-7	5	4	-9	-	-	-	-
399	2	F	50	+1	sl	12	8	-9	7	10	-9	-	-	-	P
393	2	F	52	sl	sl	10	2	-8	3	3	-11	-	-	-	P
396	2	F	49	N	sl	11	2	-11	6	5	-11	-	-	-	P
433	5	M	68	+1	+1	12	3	-10	11	4	-14	-	-	-	-
429	5	F	66	+1	+1	3	4	-12	4	3	-18	-	-	-	P
428	5	M	65	+1	+1	11	3	-9	6	5	-10	-	-	-	P
432	5	F	71	+1	+1	10	4	-10	15	3	-12	-	-	-	-
427	5	F	70	+1	+1	12	2	-8	8	5	-12	-	-	-	P
430	5	M	74	sl	sl	8	3	-7	10	6	-7	-	-	-	-
431	5	F	87	sl	sl	6	4	-7	5	4	-11	-	-	-	-
426	5	M	55	sl	+1	9	4	-7	5	9	-14	-	-	-	P
452	7	M	113	sl	sl	17	6	-12	11	7	-14	32	43	31	P
451	7	F	95	+1	+1	17	2	-12	8	4	-12	30	41	30	P
454	7	M	66	sl	sl	12	9	-6	8	7	-13	29	39	28	-
455	7	M	81	+1	+1	12	4	-8	12	6	-13	30	38	28	-
453	7	F	90	+1	+1	10	5	-7	12	7	-9	30	40	30	-
449	7	M	100	+1	+1	13	2	-6	16	4	-10	31	41	30	P
450	7	F	81	sl	sl	18	6	-7	19	2	-11	30	40	28	P
456	7	F	107	+1	+1	11	3	-6	3	5	-12	32	42	30	-
466	9	F	129	+1	+1	15	-2	-9	10	4	-12	-	-	-	P
462	9	M	152	+1	+1	16	8	-9	10	7	-4	-	-	-	-
459	9	M	136	+1	+1	20	5	-9	6	10	-13	-	-	-	-
464	9	M	140	+1	+1	16	2	-10	11	6	-6	-	-	-	P
461	9	F	117	sl	+1	7	3	-10	9	0	-10	-	-	-	-
465	9	F	146	+1	sl	18	3	-6	12	4	-14	-	-	-	P
463	9	M	134	+1	+1	15	12	-13	10	10	-7	-	-	-	P
460	9	F	135	sl	+1	10	2	-6	8	6	-7	-	-	-	-

Appendix 2 (contd)

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion degrees Left			Torsion degrees Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
485	14	F	251	+1	+1	20	-4	-10	12	0	-12	40	53	37	P
484	14	M	294	+1	+1	12	7	-6	14	2	-12	43	56	43	P
488	14	F	270	+1	+1	15	2	-10	17	-5	-12	42	54	39	-
487	14	M	260	N	s1	14	3	-10	10	4	-12	42	56	41	-
483	14	M	204	+1	+1	18	4	-10	18	2	-12	40	52	38	P
482	14	F	191	s1	+1	14	3	-12	18	4	-11	39	54	38	P
486	14	F	226	s1	+1	16	5	-12	9	8	-14	42	54	39	-
489	14	M	206	s1	s1	13	5	-10	9	2	-13	39	50	36	-
531	21	F	490	+1	+1	28	7	-9	15	4	-10	50	65	48	P
529	21	M	472	+1	+1	15	6	-5	17	7	-11	50	68	48	P
532	21	M	511	+1	+2	17	1	-8	8	3	-12	50	68	49	P
535	21	M	550	+1	+1	17	10	-7	13	2	-10	57	72	54	-
530	21	F	414	s1	+1	27	10	-9	15	9	-14	48	64	47	P
536	21	M	534	+1	+2	16	-2	-12	18	3	-10	55	74	55	-
533	21	F	520	N	+1	18	4	-8	11	2	-14	50	66	48	-
534	21	F	466	s1	+1	18	5	-8	12	2	-14	49	67	47	-
556	28	M	722	+1	+2	17	-2	-8	20	-2	-12	58	76	55	-
559	28	M	615	+1	+1	14	6	-7	10	5	-7	55	75	54	-
553	28	F	725	+1	+1	17	4	-14	10	3	-14	60	75	58	P
554	28	F	701	+1	+1	19	9	-14	12	-5	-3	60	78	58	P
558	28	F	734	+1	+1	14	4	-5	12	-1	-6	62	80	60	-
557	28	F	639	+1	+1	20	3	-6	15	9	-7	56	78	56	-
555	28	M	714	s1	+1	20	3	-10	11	2	-13	60	78	58	P
552	28	M	799	+1	+1	18	9	-9	16	4	-10	62	82	59	P
625	42	F	1455	+1	+2	11	3	-6	7	3	-8	77	104	75	-
624	42	F	1523	+1	+1	13	0	-6	7	-3	-11	77	108	78	P
621	42	M	1596	+1	+1	13	3	-13	6	3	-14	77	111	72	P
626	42	M	1718	+1	+2	17	3	-7	14	3	-10	82	106	80	-
627	42	F	1357	+1	+2	13	5	-7	5	6	-10	78	101	71	-
623	42	F	1451	+1	+1	16	8	-5	6	-6	-11	77	102	72	P
622	42	M	1705	+1	N	20	3	-5	11	-4	-10	76	104	75	P
628	42	M	1376	+2	+2	6	6	-6	8	2	-9	77	104	75	-
697	56	M	3010	+1	+2	8	-3	-5	15	-6	0	100	138	97	-
694	56	M	2960	+1	+1	11	2	-7	11	0	-7	97	134	96	P
699	56	M	2290	+1	+1	13	2	-9	10	2	-11	95	125	92	-
698	56	F	1930	+1	+1	8	8	-8	4	0	-4	90	123	83	-
692	56	F	2140	+1	+1	20	1	-8	22	-2	-6	92	130	92	P
695	56	F	1980	+1	+1	15	1	-6	10	-7	-9	88	120	85	P
696	56	M	2630	+2	+1	10	10	-8	5	-4	-12	95	128	88	-
693	56	F	2300	+1	+1	11	1	-7	6	-2	-11	93	128	88	P

Appendix 2 (contd)

Bird No.	Age (days)	Sex	Weight (grams)	Intertarsal Angulation		Torsion degrees Left			Torsion degrees Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
780	70	F	2660	s1	+1	15	1	11	5	-3	-10	95	132	92	P
782	70	M	3170	s1	+1	10	-4	-9	7	-7	-11	102	133	94	P
781	70	M	3280	s1	s1	12	0	-8	5	2	-12	105	140	97	P
784	70	F	2780	+1	+1	10	-2	-10	4	-	-10	95	130	90	-
779	70	M	3400	+1	+1	20	-2	-16	19	-3	-15	107	148	110	P
785	70	M	3500	s1	+1	18	-4	-8	11	-3	-12	106	147	11	-
783	70	F	3560	s1	+1	9	-9	-7	6	-10	-6	100	136	94	-
786	70	F	3833	s1	+1	14	1	-7	4	0	-10	109	145	109	-
1073	105	F	4120	+1	+1	15	-1	-8	6	-5	-7	104	146	98	P
1074	105	M	4625	+1	+1	9	5	-7	20	8	-7	120	165	118	P
1075	105	F	3800	s1	s1	12	3	-8	19	-2	-7	102	135	95	P
1076	105	F	4095	+1	+1	11	-2	-8	8	-1	-11	102	135	95	-
1077	105	F	3650	s1	+1	13	4	-8	12	4	-9	101	134	92	-
1078	105	M	4960	s1	+1	10	3	-9	8	3	-9	123	175	122	P
1079	105	M	3200	+1	+2	10	3	-7	11	1	-8	98	142	100	-
1080	105	M	4732	+1	s1	8	5	-8	5	3	-14	118	165	113	-
1166	140	M	5200	+1	s1	16	1	-8	13	5	-11	112	155	110	P
1167	140	M	4800	+2	+2	13	-3	-14	10	2	-15	105	142	95	P
1168	140	M	5400	+2	s1	14	-2	-14	8	0	-15	120	158	112	-
1169	140	M	5400	s1	+2	12	10	-7	11	6	-6	124	168	120	-
1170	140	M	4800	+2	+2	13	-1	-9	15	4	-12	112	160	116	-
1171	140	F	4500	+1	s1	16	0	-7	12	0	-5	96	135	92	P
1172	140	F	4300	s1	+1	13	10	-11	11	3	-13	97	136	90	P
1173	140	F	5400	+2	+2	14	10	-13	15	9	-11	120	172	118	-
1174	140	F	5000	+1	s1	18	0	-8	14	0	-6	112	162	110	-
1175	140	F	4600	+1	s1	13	-2	-9	13	3	-13	100	140	92	-

Appendix 3
Restricted Broiler fowls

Bird No.	Age (days)	Sex	Weight (grams)	Angulation		Intertarsal Torsion degrees Left				Right			Length			Processed
				L	R	F	TT	TM	F	TT	TM	F	TT	TM	
4053	28	M	477	-	-	11	7	-8	8	10	-11	55	72	54	P
4025	28	F	469	-	-	12	11	-7	5	7	-11	56	70	49	P
4086	28	M	485	-	-	7	7	-5	7	6	-5	55	70	49	-
4087	28	M	440	-	-	6	8	-9	4	9	-7	55	70	49	-
4078	28	M	587	-	-	7	7	-6	7	5	-6	58	77	55	-
4014	28	F	496	-	-	7	7	-9	6	8	-7	56	70	50	-
4004	28	F	518	-	-	6	3	-6	6	6	-8	56	72	51	-
4023	28	F	436	-	-	9	5	-6	10	6	-7	54	68	48	-
4041	42	F	610	-	-	12	10	-12	8	8	-12	67	86	60	P
4099	42	M	902	-	-	17	10	-12	10	10	-14	73	69	70	P
4058	42	M	811	-	-	7	7	-10	8	5	-10	68	90	65	-
4069	42	M	760	-	-	9	7	-7	8	4	-9	66	87	62	-
4070	42	M	868	-	-	5	12	-6	6	9	-7	70	91	66	-
4001	42	F	673	-	-	5	13	-6	7	12	-8	65	85	57	-
4035	42	F	531	-	-	4	12	-9	5	12	-9	59	80	55	-
4036	42	F	628	-	-	11	7	-8	9	5	-12	66	86	59	-
4002	56	F	912	-	-	14	13	-12	13	10	-12	74	93	68	-
4038	56	F	818	-	-	12	5	-10	14	18	-14	68	90	61	-
4039	56	F	823	-	-	9	7	-7	6	9	-9	72	95	65	-
4067	56	M	953	-	-	17	12	-10	13	13	-10	78	101	72	-
4073	56	M	869	-	-	12	10	-8	8	8	-10	72	96	68	-
4081	56	M	1092	-	-	10	11	-7	12	11	-8	78	101	74	-
4090	56	M	916	-	-	15	11	-14	12	14	-13	76	101	68	P
4046	56	F	665	-	-	13	11	-12	8	8	-13	66	90	60	P

Appendix 4

Reprints of publications containing material relevant to this thesis.

Duff,S.R.I. & Thorp.B.H. (1985) Patterns of physiological bone torsion in the pelvic appendicular skeletons of domestic fowl. Res. Vet. Sci. 39. 307-312.

Thorp.B.H. (1986) Vascular pattern of the developing proximal femur in the domestic fowl. Res. Vet. Sci. 40. 231-235.

Thorp.B.H; Lynch,M. & Duff,S.R.I. (1986) Embedding of skeletal tissue in plastic for vascular and histological study to demonstrate delayed endochondral ossification in leghorn type fowl. Res. Vet. Sci. 40. 236-240.

Research in Veterinary Science 1985, 39, 307–312

Patterns of physiological bone torsion in the pelvic appendicular skeletons of domestic fowl

S. R. I. DUFF, B. H. THORP, *Agricultural and Food Research Council Poultry Research Centre, Roslin, Midlothian EH25 9PS*

Femoral, tibiotarsal and tarsometatarsal torsion was measured in 264 domestic fowls. Birds were either a laying strain fed ad libitum, or a broiler strain fed ad libitum or a broiler strain on restricted feed. They were killed at different times up to 24 weeks of age. Femora were normally rotated externally when the transverse axis of the distal articular surface was compared with that of the proximal joint surface. Tibiotarsi were similarly rotated externally but the high incidence of internal torsion in broilers fed ad libitum suggests that internal rotation is pathological. Tarsometatarsi were normally rotated internally. Mild degrees of intertarsal valgus angulation are physiological but asymmetrical angulation and torsion between each limb of individual birds suggests that a pattern of limb dominance occurs in domestic poultry.

SEVERAL authors have identified abnormal long bone torsion (rotation) as a cause of lameness in domestic poultry (Osbaldiston and Wise 1967, Laursen-Jones 1968, Poulos et al 1978, Randall and Mills 1981, Riddell 1981). Most frequently, the condition is unilateral, with 'lateral twisting' and valgus angulation of the distal fibiotarsus being identified (Riddell 1981). Abnormal tibiotarsal rotation without angular deformity is considered to be a separate condition (Riddell 1981).

The pattern of normal, developmental torsion in the pelvic limbs of domestic fowl has not been reported and is the purpose of this study. Once physiological torsion has been determined, more useful evaluation of abnormal or pathological bone torsion can be made.

From a comparative viewpoint, postnatally the distal tibia of sheep gradually rotates internally relative to its proximal joint surface (Lanyon and Bourn 1979, Duff 1985). In children, however, torsion develops in the opposite direction, so that external tibial torsion establishes during childhood (Le Damany 1909, Nachlas 1934, Hutter and Scott 1949). It is of interest to determine the direction of tibiotarsal torsion in the domestic fowl which is also bipedal.

Materials and methods

A total of 264 birds were studied. The material was derived from three separate groups of birds (Table 1) each of which were floor reared on deep litter. Group A birds were of a layer strain. Groups B and C were

TABLE 1: Case material and experimental survival time

	Group	Number Males	Females	Diet	Survival time
Layer strain	A	48	48	0-4 weeks — starter (23% protein — 3000 kCal ME) ad lib 5-24 weeks — grower (19% protein 3000 kCal ME) ad lib	4 males and 4 females killed at days 2, 5, 7, 9, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 10 weeks, 15 weeks, 20 weeks and 24 weeks
Broiler breeding strain	B	52	52	0-4 weeks — starter (23% protein — 3000 kCal ME) ad lib 5-20 weeks — grower (19% protein 3000 kCal ME) ad lib	4 males and 4 females killed at days 0, 2, 5, 7, 9, 2 weeks, 3 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 15 weeks and 20 weeks
	C	32	32	0-2 weeks — starter (23% protein — 3000 kCal ME) ad lib 2-20 weeks — restricted grower diet (19% protein — 3000 kCal ME) adjusted as for commercial regime for breeding birds	4 males and 4 females killed at 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks, 16 weeks and 20 weeks

ME Metabolisable energy

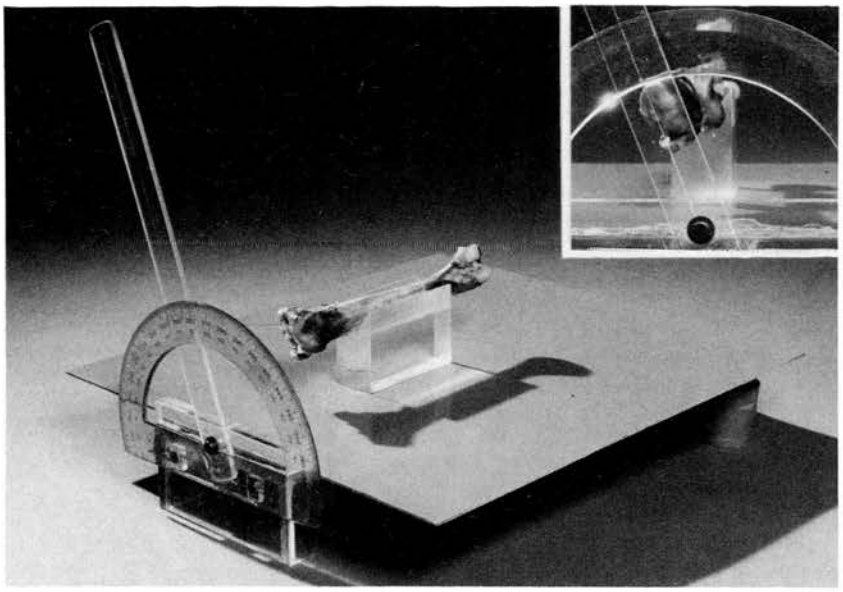


FIG 1: Apparatus used for estimates of bone torsion. The movable arm is positioned to coincide with the transverse axis of proximal and distal articular surfaces and the angular difference between each recorded

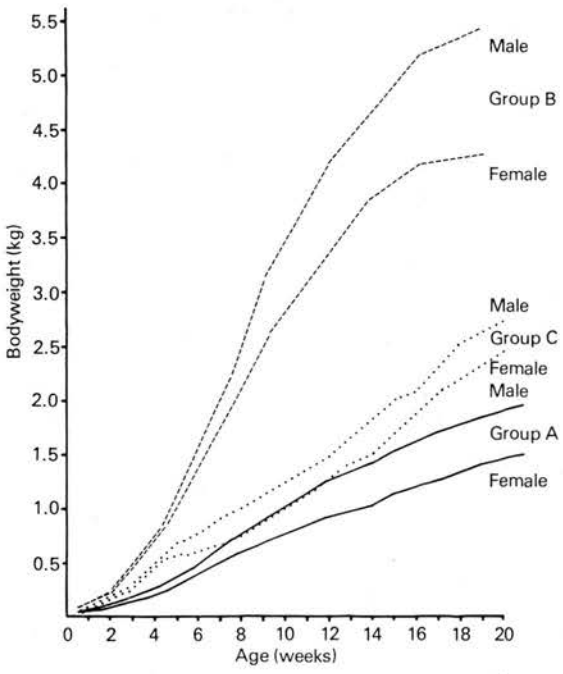


FIG 2: Bodyweight in kg plotted against age in weeks for groups A, B and C males and females

from broiler breeding lines and were of similar parentage. Although groups A and B were fed ad libitum throughout life, group C were fed a restricted ration from two weeks old according to normal commercial practice for breeding birds.

Birds were weighed at weekly intervals throughout the experimental period. Samples from each group were killed by intravenous barbiturate overdose at different times throughout the experimental period (Table 1). Birds from group B which were identified as lame are not included in this report. After death, angular deviation of limbs was recorded before dissection of each major long bone (femur, tibiotarsus and tarsometatarsus).

Torsional measurements were made by comparing the transverse axes of the proximal and distal articular surfaces of each long bone (Fig 1). All estimates of bone torsion were duplicated by independent assessment by both authors.

Results

Liveweight gain

The average liveweight gain of male and female birds of each group is given in Fig 2. Birds were killed for autopsy throughout the experimental period, so the number of birds remaining and contributing to the mean bodyweight decreased as the experiment progressed.

Limb angulation

In most birds examined, mild valgus angulation occurred at the intertarsal joint.

In group A (laying strain birds) intertarsal valgus was identified in over 90 per cent of birds and most frequently the angulation ranged between 5° and 10°. In some birds, aged four weeks or more when killed, up to 15° angulation was identified. Intertarsal valgus was greater in the right pelvic limb than the left in 56 per cent of group A birds and greater in the left limb in 10 per cent. Intertarsal valgus was judged to be equal in both limbs in the remaining 34 per cent of cases.

In group B (broiler strain birds) intertarsal valgus was similarly identified in over 90 per cent of birds. In two limbs intertarsal varus of 5° and 10° was present. Intertarsal valgus was most frequently between 5° and 15° with 15° angulation being identified as early as two days of age. In approximately 18 per cent of cases aged three weeks or more, up to 20° intertarsal valgus was judged to be present. Intertarsal valgus was usually greater in the right pelvic limb (62 per cent of cases) than in the left (15 per cent) but was of equal severity in each limb in 23 per cent of group B birds.

Limb angulation was not recorded for group C (broiler strain birds).

Estimates of bone torsion

Estimates of bone torsion were made independently by both authors and discrepancies rarely exceeded 4°. In most instances agreement of estimates within 2° was obtained. When estimates differed by more than 4°, measurements were repeated until agreement was achieved.

Distal femora were normally rotated externally relative to their proximal joint surface (Table 2) and in at least 97 per cent of all cases between 2° and 20° external torsion was measured.

Similarly distal tibiotarsi were normally externally rotated but internal torsion was present in a proportion of broilers (Table 2). Tibiotarsal torsion was estimated at between 0° and 15° external rotation in

TABLE 2: Estimates of bone torsion (arithmetic mean and range)

	Age (days)	Femoral torsion Mean	Femoral torsion Range	Tibiotarsal torsion Mean	Tibiotarsal torsion Range	Tarsometatarsal torsion Mean	Tarsometatarsal torsion Range
Group A	2	4·1 E	0 → 7°E	3·6 E	6°I → 8°E	5·8 I	3°I → 9°I
	5	7·5 E	4°E → 12°E	6·7 E	3°E → 12°E	7·2 I	4°I → 12°I
	7	8·4 E	5°E → 14°E	7·8 E	2°E → 12°E	7·1 I	5°I → 10°I
	9	9·1 E	6°E → 15°E	7·3 E	3°E → 12°E	8·5 I	6°I → 13°I
	14	13·1 E	10°E → 18°E	8·1 E	2°E → 12°E	7·7 I	5°I → 12°I
	21	13·9 E	7°E → 26°E	10·0 E	3°E → 19°E	9·1 I	7°I → 13°I
	28	13·0 E	7°E → 20°E	9·6 E	2°E → 16°E	10·9 I	7°I → 16°I
	42	13·8 E	5°E → 22°E	8·8 E	3°E → 14°E	11·1 I	8°I → 15°I
	70	11·5 E	5°E → 18°E	7·5 E	2°E → 16°E	9·7 I	5°I → 17°I
	105	9·2 E	5°E → 16°E	5·1 E	0 → 8°E	10·3 I	5°I → 12°I
	140	4·9 E	0 → 9°E	3·9 E	1°E → 10°E	7·9 I	6°I → 11°I
	168	5·8 E	2°E → 13°E	7·9 E	2°E → 17°E	9·9 I	6°I → 15°I
Group B	0	9·6 E	4°E → 16°E	5·1 E	8°I → 13°E	9·8 I	5°I → 13°I
	2	9·4 E	3°E → 15°E	5·1 E	0 → 10°E	9·1 I	6°I → 15°I
	5	8·4 E	3°E → 15°E	4·1 E	2°E → 9°E	10·5 I	7°I → 18°I
	7	12·4 E	3°E → 18°E	3·7 E	6°I → 9°E	10·0 I	6°I → 14°I
	9	12·1 E	6°E → 20°E	5·0 E	2°I → 12°E	9·1 I	4°I → 14°I
	14	14·3 E	9°E → 20°E	2·6 E	5°I → 8°E	11·1 I	6°I → 13°I
	21	16·3 E	8°E → 28°E	4·6 E	2°I → 10°E	10·0 I	5°I → 14°I
	28	15·3 E	10°E → 20°E	3·8 E	2°I → 9°E	8·3 I	3°I → 14°I
	42	10·8 E	5°E → 20°E	2·6 E	4°I → 8°E	7·9 I	6°E → 14°I
	56	11·2 E	5°E → 22°E	0	7°I → 10°E	7·4 I	0 → 12°I
	70	10·5 E	4°E → 19°E	2·7 I	10°I → 2°E	10·1 I	7°I → 16°I
	105	11·7 E	5°E → 20°E	1·8 E	5°I → 8°E	8·4 I	7°I → 14°I
	140	13·2 E	8°E → 18°E	2·0 E	5°I → 10°E	10·4 I	5°I → 15°I
Group C	7	5·4 E	2°E → 8°E	3·0 E	3°I → 7°E	8·3 I	4°I → 11°I
	14	5·8 E	4°E → 11°E	4·6 E	3°E → 11°E	8·4 I	7°I → 11°I
	28	7·9 E	5°E → 12°E	5·8 E	0 → 11°E	9·8 I	6°I → 12°I
	42	8·6 E	2°E → 13°E	6·7 E	1°E → 14°E	11·9 I	8°I → 15°I
	56	9·6 E	3°E → 15°E	6·1 E	3°I → 14°E	11·6 I	8°I → 14°I
	84	6·8 E	0 → 10°E	8·4 E	1°E → 15°E	9·9 I	7°I → 15°I
	112	5·9 E	2°I → 17°E	6·2 E	4°I → 15°E	10·1 I	5°I → 17°I
	140	8·3 E	4°E → 13°E	5·6 E	2°I → 13°E	9·8 I	5°I → 17°I

E External torsion (rotation) of distal, relative to proximal, articular surface
I Internal torsion (rotation) of distal, relative to proximal, articular surface

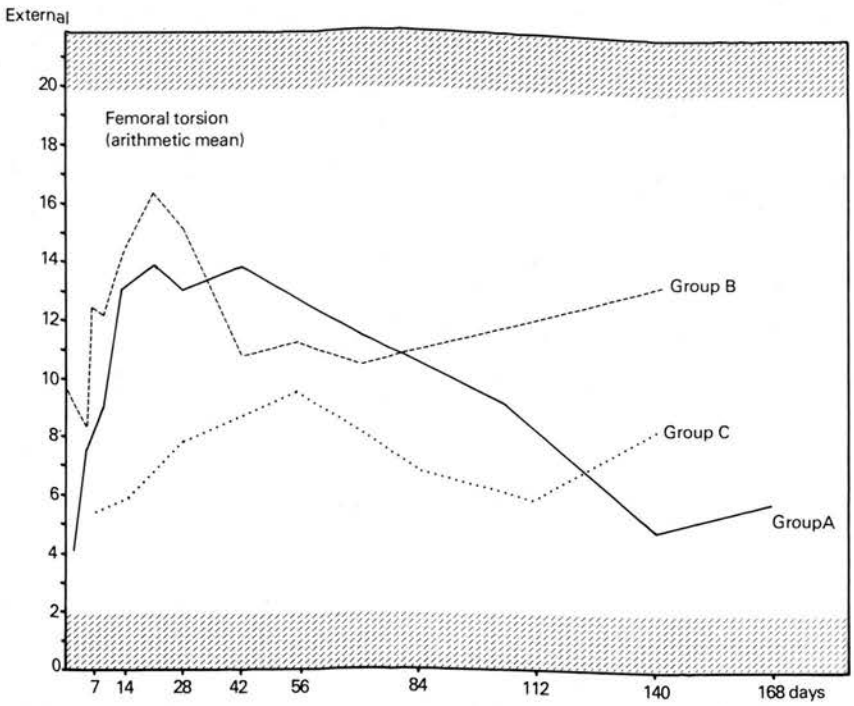


FIG 3: Arithmetic mean of femoral torsion plotted against age. The unshaded range contains 97 per cent of all individual measurements

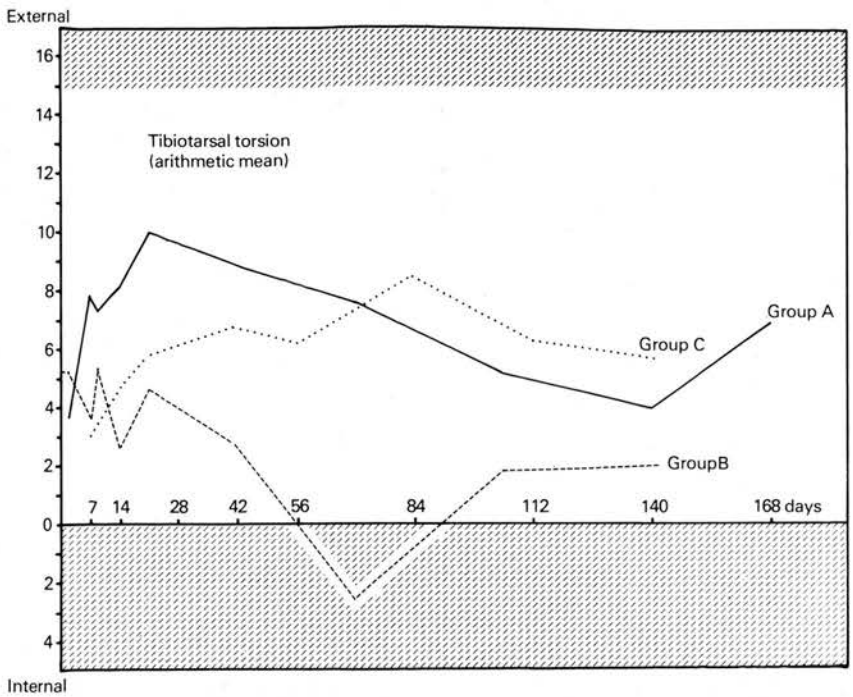


FIG 4: Arithmetic mean of tibiotarsal torsion plotted against age. The unshaded range contains almost 95 per cent of groups A and C measurements but only 66 per cent of estimates in group B birds

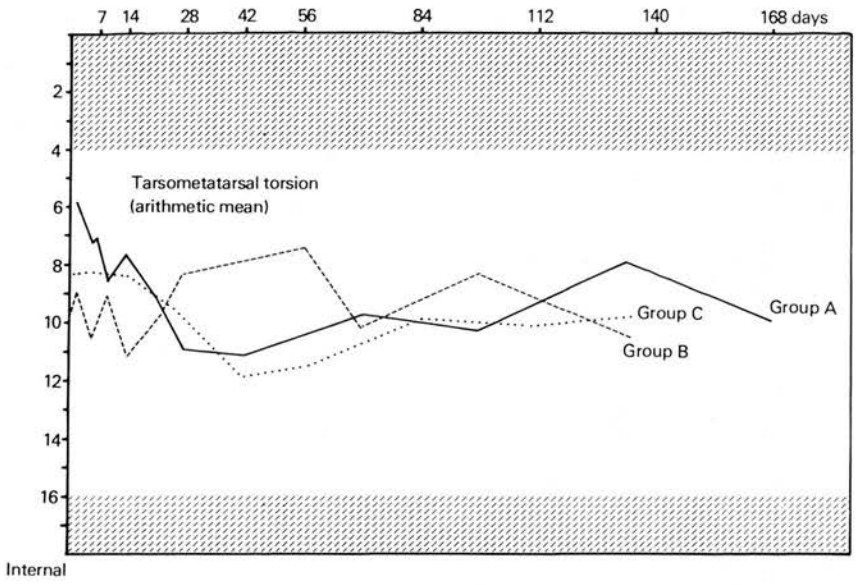


FIG 5: Arithmetic mean of tarsometatarsal torsion plotted against age. The unshaded area contains 97 per cent of all individual measurements

97 per cent of group A birds but in 94 per cent of group C and only 66 per cent of group B birds. Internal tibiotarsal torsion was identified in group A (two unilateral cases), group B (43 limbs, bilateral 12 birds, unilateral 19 birds) and in group C (seven limbs, bilateral one bird, unilateral five birds).

Distal tarsometatarsi were rotated internally relative to their proximal joint surface in all but two cases (group B) which showed 0° and 6° external torsion respectively. In at least 97 per cent of all cases between 4° and 16° internal torsion was measured.

The pattern of torsion in groups A, B and C birds is given in Figs 3, 4 and 5 with the arithmetic mean of torsional estimates being plotted against age.

Asymmetry of torsional estimates between the limbs of individual birds was frequently observed (Table 3). External femoral torsion was normally greater in the left pelvic limb whereas internal tarsometatarsal torsion was more often greater in the right limb. No clear pattern emerged for external tibiotarsal torsion. When internal tibiotarsal torsion was recorded, the right pelvic limb was affected to a greater extent than the left limb in group B birds. Torsional differences between right and left bones in individual birds ranged from 1° to 15° with mean values of 3° or 4° depending on the long bone being considered.

TABLE 3: Asymmetry of torsion between limbs of individual birds

Long bone torsion	Group	Percentage of cases in which bone torsion asymmetry occurred R > L	L > R	R = L
External femoral torsion	A	12	82	6
	B	19	75	6
	C	13	70	17
External tibiotarsal torsion	A	44	39	17
	B	43	50	7
	C	32	38	30
Internal tarsometatarsal torsion	A	70	18	12
	B	71	18	11
	C	59	24	17

Discussion

The purpose of this study was to elucidate certain aspects of normal pelvic limb development in domestic fowl. Broiler fowl, however, cannot be considered normal as selection for certain production traits, with 'little consideration to function and health', has increased their susceptibility to skeletal disease (Reiland et al 1978). By contrast, skeletal development in slower growing, layer strain fowl is considered to be more normal (Reiland et al 1978). In this study, therefore, group A (laying strain) would be expected to demonstrate a more normal pattern than group B (broilers). If restricted liveweight gain is sufficient to modify broiler skeletal development, then group C would be intermediate between groups A and B. Fig 1 demonstrates that feed restriction in group C birds has resulted in a pattern of liveweight gain closer to that of laying strain birds (group A) than ad libitum fed broilers (group B).

The results suggest that intertarsal valgus angulation of 10° or less is normal and should be regarded as physiological limb angulation. Valgus angulation of 20° or more and intertarsal varus angulation should be regarded as abnormal. The finding that the right pelvic limb more frequently showed greater degrees of valgus angulation than the left limb in both group A and B birds suggests that a pattern of limb dominance may occur in fowls. Other studies have suggested that limb dominance occurs in man (Lowrance and Latimer 1957, Latimer and Lowrance 1965, Chhibber and Singh 1970, Pande and Singh 1971) and other mammals (Singh 1971).

The method of estimating bone torsion was remarkably reliable. Measurements of ovine tibial torsion employing the same method (Lanyon and Bourn 1979) rarely produced more than 3° inter-observer variation.

In birds, the range of femoral torsion was quite large but was usually external (femoral retroversion). Similarly studies on human femora reveal wide variation in torsional patterns both in fetuses and adults (Kingsley and Olmstead 1948, Brouwer 1981). It is thought that human femora gradually derotate in an external direction during childhood (Kingsley and Olmstead 1948) and avian femora may similarly acquire increased retroversion during the period of rapid growth (Fig 3). Of comparative interest is the fact that ovine femora normally demonstrate femoral anteversion and thus internal torsion (Duff 1985).

Normal, developmental tibial torsion in sheep is internal (Lanyon and Bourn 1979, Duff 1985) whereas in children external tibial torsion develops (Le Damany 1909, Nachlas 1934, Hutter and Scott 1949). In birds, which are also bipedal, external tibiotarsal torsion is physiological but internal rotation is probably abnormal. Internal tibiotarsal torsion was identified in one third of broilers fed ad libitum but in only 6 per cent of broilers on restricted diets. It is possible that internal tibiotarsal torsion is a consequence of altered long bone growth resulting from disturbed endochondral ossification. Alternatively, limb functioning may be important, as broilers are known to be relatively lethargic when compared with laying strain birds (Reiland et al 1978). Duff (1985) suggested that normal limb function is an essential prerequisite for establishment of normal developmental torsion in sheep.

Tarsometatarsi in birds normally develop internal torsion and external torsion is probably abnormal.

The finding that torsional estimates frequently differed in the two limbs of individual birds further suggests that a pattern of limb dominance occurs in domestic poultry.

References

BROUWER, K. J. (1981) *Acta Orthopaedica Scandinavica*, Supplementum 195

CHHIBBER, S. R. & SINGH, I. (1970) *Journal of Anatomy* **106**, 553–556

DUFF, S. R. I. (1985) *Journal of Comparative Pathology* (In press)

HUTTER, C. G. & SCOTT, W. (1949) *Journal of Bone and Joint Surgery* **31A**, 511–518

KINGSLEY, P. C. & OLMSTEAD, K. L. (1948) *Journal of Bone and Joint Surgery* **30A**, 745–751

LANYON, L. E. & BOURN, S. (1979) *Journal of Bone and Joint Surgery* **61A**, 263–273

LATIMER, H. B. & LOWRANCE, E. N. (1965) *Anatomical Record* **152**, 217–224

LAURSEN-JONES, A. P. (1968) *Veterinary Record* **83**, 597

LE DAMANY, P. (1909) *Journal de l'Anatomie et de la Physiologie (Paris)* **45**, 598–615

LOWRANCE, E. N. & LATIMER, H. B. (1957) *American Journal of Anatomy* **101**, 445–459

NACHLAS, I. W. (1934) *Archives of Surgery* **28**, 909–919

OSBALDISTON, G. W. & WISE, D. R. (1967) *Veterinary Record* **80**, 320–322

PANDE, B. S. & SINGH, I. (1971) *Journal of Anatomy* **109**, 457–459

POULOS, P. W., REILAND, S., ELWINGER, K. & OLSSON, S-E. (1978) *Acta Radiologica, Supplementum* **358**, 229–275

RANDALL, C. J. & MILLS, C. P. J. (1981) *Avian Pathology* **10**, 407–431

REILAND, S., OLSSON, S.-E., POULOS, P. W. & ELWINGER, K. (1978) *Acta Radiologica, Supplementum* **358**, 277–298

RIDDELL, C. (1981) *Advances in Veterinary Science and Comparative Medicine* **25**, 277–310

SINGH, I. (1971) *Journal of Anatomy* **109**, 271–275

Received for publication October 25, 1984

Research in Veterinary Science 1986, 40, 236–240

Embedding of skeletal tissue in plastic for vascular and histological study to demonstrate delayed endochondral ossification in Leghorn type fowl

B. H. THORP, M. LYNCH, S. R. I. DUFF, *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

A method is described which enables visualisation of the blood supply in developing avian long bones, followed by the preparation of undecalcified histological sections from the same material. The circulatory system was perfused with a solution of fixative, dye and barium sulphate. The skeletal tissue was cleared in plastic resin before embedding and tissue blocks were cut into 1 mm slabs. The vascular canals were then examined with a dissecting microscope. Slabs were re-embedded in resin and 5 μm sections cut for routine undecalcified histological staining. Focal areas of delayed endochondral ossification were demonstrated in slabs prepared from the proximal ends of femora of White Leghorns. These lesions are considered a less severe form of the dyschondroplastic condition occurring in broilers.

VISUALISATION of the blood supply in developing long bone followed by the preparation of undecalcified histological sections of the same tissue is invaluable in the study of local and regional vascularity. Investigative techniques have attempted to achieve this. One method has been to perform microangiography on perfused mammalian long bones. Subsequently histological sections were prepared from the same specimens (Kelly et al 1959, Sevitt 1964). Brookes and Lauden (1964) studied cleared specimens of perfused long bones using the Spalteholz (1924) technique, and looked at grossly similar material histologically. Another method has been to prepare thick and thin slabs from a tissue block and to examine thick slabs by the Spalteholz clearing method and thin sections by histological methods (Spira et al 1963, Trueta and Buhr 1963).

In recent years plastic resins have been used as embedding media for the production of undecalcified sections of skeletal tissues (Difford 1974, Ellis 1981). Epoxy resins have been used for embedding Spalteholz cleared specimens for subsequent histological study (Reinhold et al 1983).

The investigation of lesions caused by defective endochondral ossification is facilitated by the examination of serial sections. Other methods such as gross radiological study or random sectioning makes the detection of minor lesions difficult. Dyschondroplastic lesions involving the avian proximal femur have been documented extensively in broilers (Riddell et al 1983, Duff 1984, 1985). Reiland et al (1978) in a comparison of skeletal development in broilers and Leghorns found no evidence of dyschondroplastic lesions occurring in the Leghorn.

Using the technique described by Mawhinny and Ellis (1983), Polymaster resin (Polymaster 1209AC; Bondaglass-Voss) has been successfully applied in the production of undecalcified bone sections. The purpose of this report is to describe the use of Polymaster resin to clear blocks of perfused avian skeletal tissue. In addition, histological sections were prepared from slabs used for vascular studies. The preparation of material by this technique demonstrated focal areas of delayed endochondral ossification occurring in White Leghorns.

Materials and methods

As part of an investigation into growth and vascularity of the appendicular skeleton in domestic poultry, 104 birds were studied. The birds were injected with a solution of heparin before being killed by intravenous barbiturate overdose. Aortic catheterisation was performed and the pelvic limbs perfused with a solution of 10 per cent buffered neutral formalin containing 17·5 per cent barium sulphate, 3·5 per cent sodium citrate and 2 per cent Berlin blue. Perfusion was maintained at a constant pressure of 150 mm Hg until the skin overlying the pelvic limbs had turned blue. After perfusion, the pelvic appendicular skeleton was dissected. Bone extremities were either fixed in 70 per cent ethanol and then transferred to absolute ethanol or fixed directly in absolute ethanol.

The technique used in the preparation of Polymaster resin infiltrated tissue blocks was based on the method of Mawhinny and Ellis (1983). Before pro-

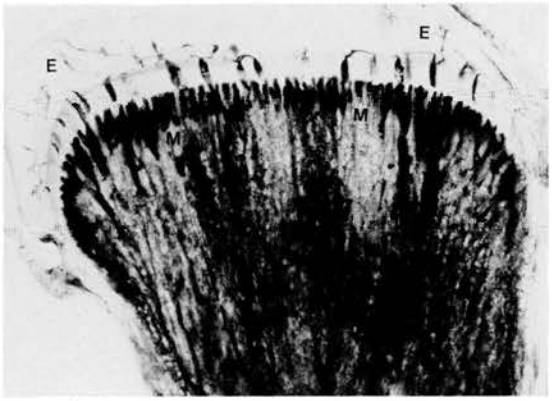


FIG 1a: Proximal femur (1 mm slab) demonstrating epiphyseal (E) and metaphyseal (M) blood vessels. ×4

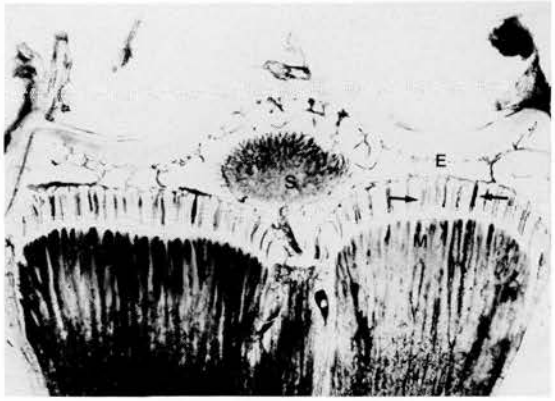


FIG 1b: Proximal tarsometatarsus (1 mm slab) demonstrating epiphyseal (E) vessels, metaphyseal (M) vessels, penetrating epiphyseal vessels (arrowed) and the secondary (tarsal) ossification centre (S). ×4

cessing, some of the larger specimens were halved in the coronal plane using a band saw. Due to the large size of some of the specimens the infiltration times, with resin and resin/cellosolve, were increased by up to 96 hours.

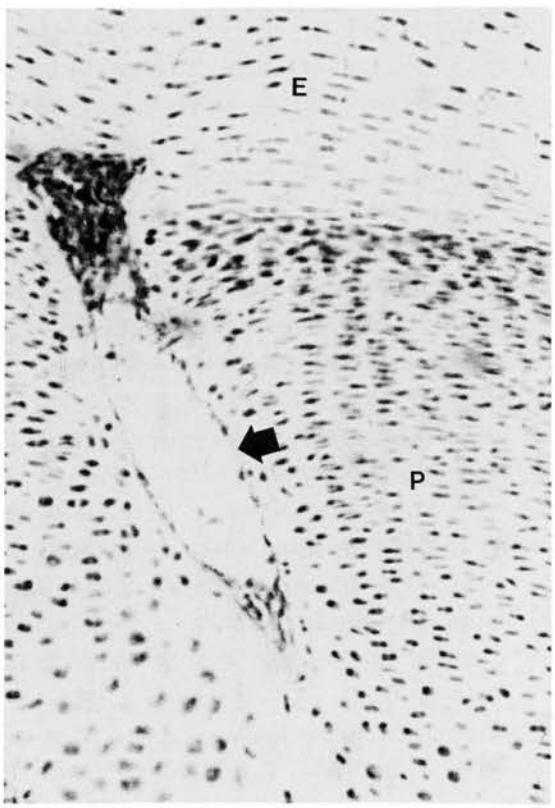


FIG 2a: Physis (growth plate) (P) from proximal femur containing a penetrating epiphyseal vessel (arrowed); epiphysis (E), H&E ×60

The blocks of tissue embedded in resin were trimmed with a band saw. One millimetre thick slabs were cut on a precision annular saw (Microslice 2; Metals Research). Slabs were examined with a binocular dissecting microscope, by transmitted light. Details of tissue vasculature were recorded by a 35 mm camera attached to the microscope.

Slabs were re-embedded in Polymaster resin and thin (5 μm) histological sections were cut on a heavy duty microtome (Polycut; Reichart-Jung) using a tungsten carbide knife. A variety of routine stains was used to stain the sections.

Results

When the tissue was placed directly in absolute ethanol gross distortion and shrinkage of the cartilage

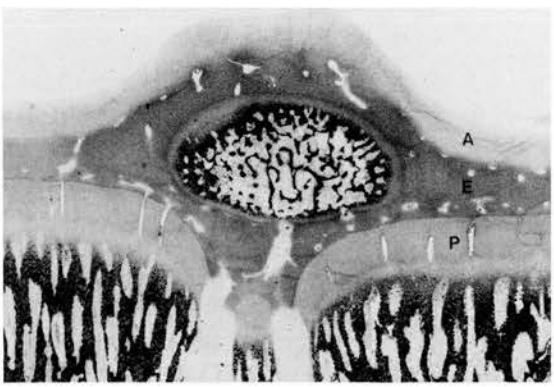


FIG 2b: Proximal tarsometatarsus with the secondary (tarsal) ossification centre surrounded by epiphyseal hyaline cartilage. Calcified tissue is a dense black. Articular (A) epiphyseal (E) and physeal (growth plate) (P) cartilage are demonstrated. Von Kossa ×7

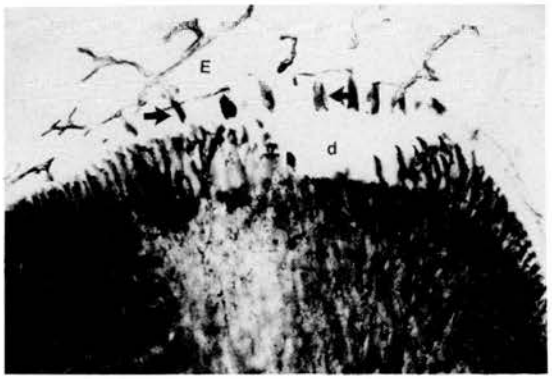


FIG 3a: Femoral head with metaphyseal defect (d) from a 10-week-old Leghorn. Penetrating epiphyseal vessels (arrowed) enter the physis (growth plate) from the epiphysis (E). 1 mm slab ×4

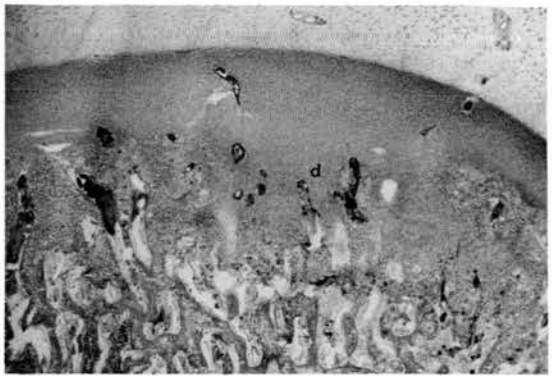


FIG 3b: The metaphyseal defect (d) from Fig 3a which is an area of retained physeal cartilage consisting of avascular hypertrophic chondrocytes. H&E ×17

occurred. This did not happen when 70 per cent ethanol was used as a fixative before absolute ethanol.

During processing in cellosolve/resin mixtures premature polymerisation of the Polymaster occasionally occurred. This was due to the formation of peroxides by the cellosolve which acted as a resin catalyst.

Tissue impregnation by Polymaster resin was assessed by the degree of tissue clearing. The cartilaginous epiphysis cleared sufficiently to allow visualisation of the perfused vascular channels. The haemopoietic marrow tissue in the metaphysis was poorly cleared by the resin. When the epiphyseal tissue cleared it was found that there was sufficient resin infiltration to proceed with the next stage in processing.

A drop of immersion oil was placed on the surface of the 1 mm sections when they were examined with the binocular microscope greatly improving visualisation of the vascular channels. The binocular microscope allowed stereoscopic examination of the tissue. In Fig 1a the epiphyseal and metaphyseal vessels of a proximal femur are demonstrated. Fig 1b is of a proximal tarsometatarsus where the vessels associated with the developing tarsal ossification centre are apparent. These slabs of tissue were re-embedded to produce 5 μm sections seen in Figs 2a and 2b. The histological sections demonstrate the preservation of detail using this technique.

A small number of proximal femurs demonstrated focal areas of delayed endochondral ossification. Two types of lesion were identified. In the mid-femoral head thickening of physeal cartilage occurred, causing avascular cartilage to extend into the metaphysis (Fig 3a). Histologically (Fig 3b) these lesions were characterised by an increased thickness of the transitional zone of chondrocytes. This type of lesion occurred in two femoral heads from a group of eight birds of 10 weeks of age. In a 20-week-old specimen a lesion involving the anterior portion of the trochanter occurred. There was failure of metaphyseal vessels to penetrate the hyaline cartilaginous epiphysis (Fig 4a). In sections examined from both types of lesion epiphyseal vascular canals were patent. Metaphyseal vessels appeared normal, but there was delayed progression in the regions of delayed endochondral ossification (Fig 3b and 4b).

Discussion

The use of 70 per cent ethanol as a fixative before absolute ethanol prevented rapid dehydration of the tissue and reduced the shrinkage of the cartilaginous skeletal components.

To reduce the risk of premature resin polymerisation due to the formation of cellosolve peroxides a number of precautions can be taken; solutions should be continually agitated on rollers, be kept cool and out of direct sunlight and be changed regularly.

The method described uses a procedure which has been shown to preserve histological detail. The modification of the process by perfusion and the cutting of 1 mm slabs allows the tissue vasculature to be studied. Previous methods have the disadvantage of either not allowing subsequent histological examination of perfused tissue, or in loss of cellular detail as a result of processing.

The preparation of thin (1 mm) slabs of perfused plastic embedded skeletal tissue aids the detection of vascular associated lesions. The femoral head changes described are considered to be characteristic of avian dyschondroplasia (Poulos et al 1978, Duff 1984). The cranial trochanteric lesion described is typical of retained hyaline cartilage and can be termed osteochondrosis (Duff 1985a); arguably such a lesion

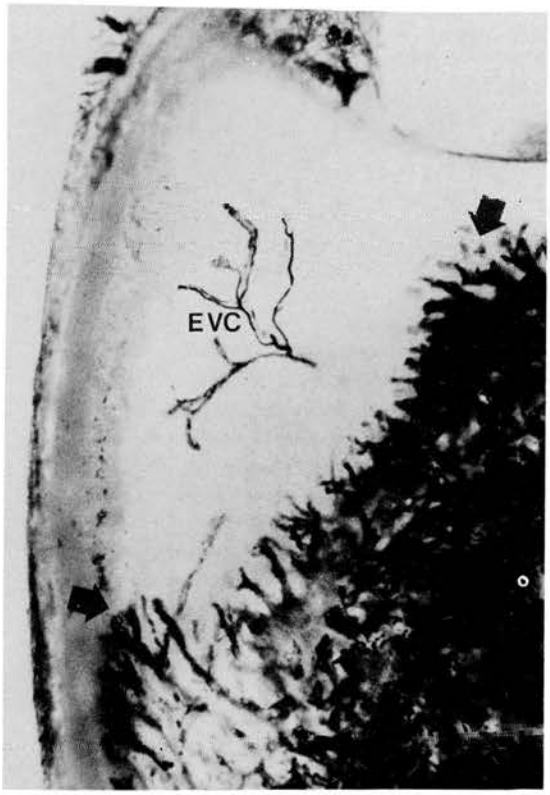


FIG 4a: Epiphyseal hyaline cartilage retention in a femoral trochanteric site. An epiphyseal vascular canal (EVC) is present in the trochanter metaphyseal vessels (arrowed) are penetrating the epiphysis around the margins of the area of cartilage retention. 1 mm slab × 6

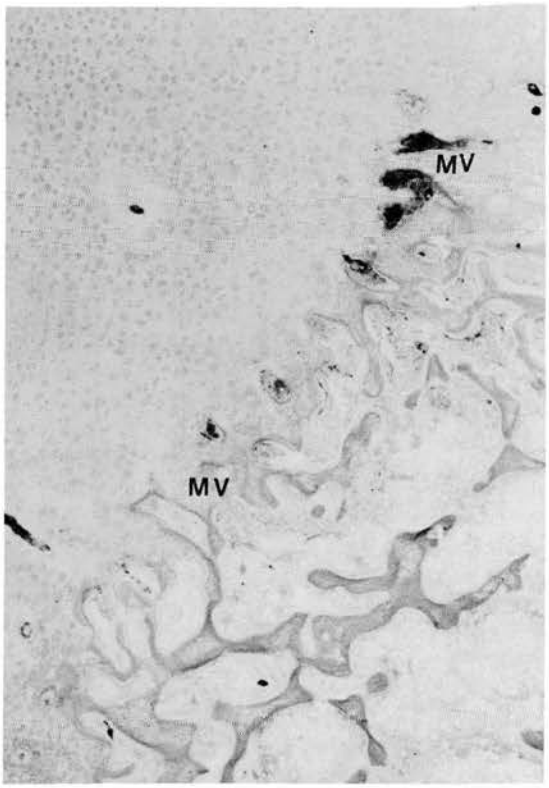


FIG 4b: Delayed progression of metaphyseal vessels (MV) in region of epiphyseal hyaline cartilage retention. H&E × 15

involving articular epiphyseal complexes is a form of dyschondroplasia (Hill et al 1984). Similar but more extensive lesions of the proximal femur have been associated with lameness in broilers (Duff 1984, 1985b). Clinically lameness was not evident in any of the birds in this experiment. It is suggested that the normal Leghorn fowl can repair small areas of aberrant endochondral ossification as a normal physiological process, while in the broiler, with its modified genotype, increased growth rate, size and weight, more extensive and severe lesions might occur. The relatively normal skeletal growth in the Leghorn is emphasised by the low incidence of abnormal endochondral ossification in this report.

This technique has been used in this laboratory with equal success in the study of mammalian skeletal tissues.

Acknowledgements

We thank Mr W. H. B. Mawhinny of the department of pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne, for his help and advice on the use of Polymaster resin as an embedding medium.

References

BROOKES, M. & LAUDEN, D. N. (1964) *Journal of Bone and Joint Surgery* **46B**, 336–345

DIFFORD, J. (1974) *Medical Laboratory Technology* **31**, 79–81

DUFF, S. R. I. (1984) *Research in Veterinary Science* **37**, 293–302

DUFF, S. R. I. (1985a) *Journal of Comparative Pathology* **95**, 363–371

DUFF, S. R. I. (1985b) *Journal of Comparative Pathology* **95**, 384–392

ELLIS, H. A. (1981) Recent Advances in Histopathology. Eds P. P. Antony, R. N. M. Macsween. Edinburgh, Churchill-Livingstone. pp 185–202

HILL, M. A., RUTH, G. R., HILLERY, H. D. & HANSGEN, D. C. (1984) *American Journal of Veterinary Research* **45**, 903–916

KELLY, P. K., PETERSON, L. F. A. & JANIS, J. M. (1959) *Staff Meetings of the Mayo Clinic* **34**(11), 274–383

MAWHINNY, W. H. B. & ELLIS, H. A. (1983) *Journal of Clinical Pathology* **36**, 1197–1199

POULOUS, P. W., REILAND, S., ELWINGER K. & OLSSON, S. E. (1978) *Acta Radiologica* Supplement **358**, 229–276

REILAND, S., OLSSON, S. E., POULOS, P. W. & ELWINGER, K. (1978) *Acta Radiologica* Supplement **358**, 277–298

REINHOLD, H. S., HOPEWELL, J. W. & VAN RIJSOORT, A. (1983) *International Journal of Microcirculation* Clin Expt 2, 47–52

RIDDELL, C., KING, M. W. & GUNASEKERA, K. R. (1983) *Avian Diseases* **27**, 980–991

SEVITT, S. (1964) *Journal of Bone and Joint Surgery* **46**, 270–296

SPALTEHOLZ, W. (1924) *Handbuch der Biologischen Arbetsmethoden* **9**, 409–436

SPIRA, E., FARIN, I. & KARPLUS, H. (1963) *Journal of Anatomy London* **97**, 255–258

TRUETA, J. & BUHR, A. J. (1963) *Journal of Bone and Joint Surgery* **45B**, 572–581

Accepted July 9, 1985

Research in Veterinary Science 1986, 40, 231–235

Vascular pattern of the developing proximal femur in the domestic fowl

B. H. THORP, *Agricultural and Food Research Council's Poultry Research Centre, Roslin, Midlothian EH25 9PS*

The vascular pattern of the proximal femur and its importance in the aetiology of coxofemoral conditions has been established in many species, including man. In the avian femur, vascular lesions have been associated with pathological conditions. Ninety-six laying strain chicks were reared from day-old until 20 weeks. Birds were killed throughout the growth period and specimens were prepared for study. The origin and nature of epiphyseal vascular canals was established. Three principal stages of development occurred, namely growth plate formation, the growth period and the cessation of growth. Transphyseal vessels are described in the day-old chick to which a nutritive function is ascribed. Anastomosis did not occur between epiphyseal vascular canals. The epiphyseal vascular canals are grouped according to their source.

THE development of the vascular pattern of the femoral head in man was established by Trueta (1957). He also associated age specific developmental orthopaedic diseases with this changing pattern of vascular supply. Other authors (Hulth 1958, Catto 1965, Theron 1977) have implicated the increased susceptibility of the femoral head to vascular compromise as a cause of clinical disease.

The canine coxofemoral joint was studied by Kaderly et al (1983), who emphasised the importance of the vascular supply to the understanding of vascular impairment in relation to clinical conditions.

Levene (1964) studied epiphyseal cartilage canals and found a species specific pattern but degrees of individual variation within each species.

The vasculature of the proximal femur in domestic fowls has not been investigated. Vascular occlusion of epiphyseal cartilage canals in association with pathological lesions were recognised by Duff (1984a), the medial capitus and lateral aspect of the proximal femur were more susceptible to vascular occlusion. Duff (1984b) also discussed the importance of thrombosis and canal occlusion in the pathogenesis of femoral head epiphyseal infarction.

An attempt was made to establish the pattern of proximal femoral vasculature during growth in the domestic fowl.

Materials and methods

Ninety-six chicks (48 male and 48 female) derived from a White Leghorn strain were reared from day-old, in deep litter floor pens. The birds were fed ad libitum a standard commercial 'starter' ration until four weeks of age, followed by a 'grower' ration.

Eight birds, four from each group, were killed at day old. Thereafter similar groups of birds were killed at two days, five days, seven days, 10 days, two weeks, three weeks, four weeks, six weeks, 10 weeks, 15 weeks and 20 weeks. The birds were injected with heparin then killed with an overdose of barbiturates. Perfusion of the pelvic appendicular skeleton followed by the preparation of 1 mm Polymaster embedded slabs of the proximal end of the femur was performed using the method described by Thorp et al (1986). After binocular microscopic study selected slabs were prepared for histological examination.

In a small number (less than 5 per cent) of femurs there were abnormalities of endochondral ossification. These are reported separately (Thorp et al 1986).

Results

The epiphyseal cartilage contained epiphyseal vascular canals (EVCs), which branched through the cartilage. The EVCs terminated either in the growth plate cartilage as penetrating epiphyseal vessels (PEVs) or in the epiphyseal cartilage as blind ending capillary loops. All EVCs were end arterial systems; there were no vascular connections between individual arborising EVCs.

The EVCs could be divided into groups according to their origin. In individuals of the same age there was a similar pattern in the area supplied by an EVC or group of EVCs.

EVCs originated from: (1) the perichondrial ring; (2) the intracapsular retinacular tissue; (3) the joint capsule and associated connective tissue; (4) the extra-

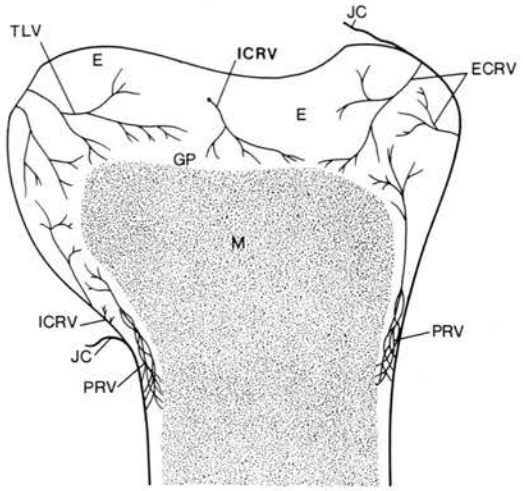


FIG 1: Coronal section of the avian proximal femur, the epiphyseal vascular canals and their sites of origin. E Epiphyseal cartilage; ECRV Originating from extracapsular retinacular vessels; GP Growth plate; ICRV Originating from intracapsular retinacular vessels; JC Joint capsule; M Metaphysis; PRV Perichondrial ring vessels; TLV Originating from teres ligament vessels

capsular retinacular tissue; (5) the teres ligament (Fig 1).

The perichondrial ring encircled the proximal femoral growth plate. The principal vascular supply to the perichondrial ring was from the mid-lateral, mid-cranial and mid-caudal aspects of the femur. It was from these three regions that the main perichondrial EVCs originated. The caudal perichondrial ring vessels formed two to three EVCs which supplied the region from the caudal trochanter to the caudolateral capitus (Fig 2, Fig 3i). The lateral femoral perichondrial ring vessels formed EVCs which supplied the trochanter (Fig 3i and Fig 4). The cranial perichondrial ring vessels only rarely formed EVCs, they were seen most frequently in day-old and six- to 10-week-old birds. These EVCs did not form PEVs.

The intracapsular retinacular tissue contained blood vessels. The retinacular blood vessels originated from the joint capsule and perichondrial ring. Vessels from the retinaculum of the cranial capitus extended on to the lateral aspect of the capitus. EVCs from the retinacular vessels supplied PEVs to the periphery of the capitus and the cranial and caudal trochanter (Fig 3ii).

The retinacular vessels of the craniolateral capitus were supplied by a large vessel, originating from extra capsular soft tissue. A branch from this vessel (Fig 5) continued from the retinacular vessels to supply the craniolateral capitus area (a) in Fig 3iii.

FIG 2: Slab from the right proximal femur of a 14-day-old bird. The caudal perichondrial ring vessels (A), between the femoral head and trochanter, supplies EVCs (arrowed) to the cartilage of the femoral head and cartilage of the trochanter. ×11

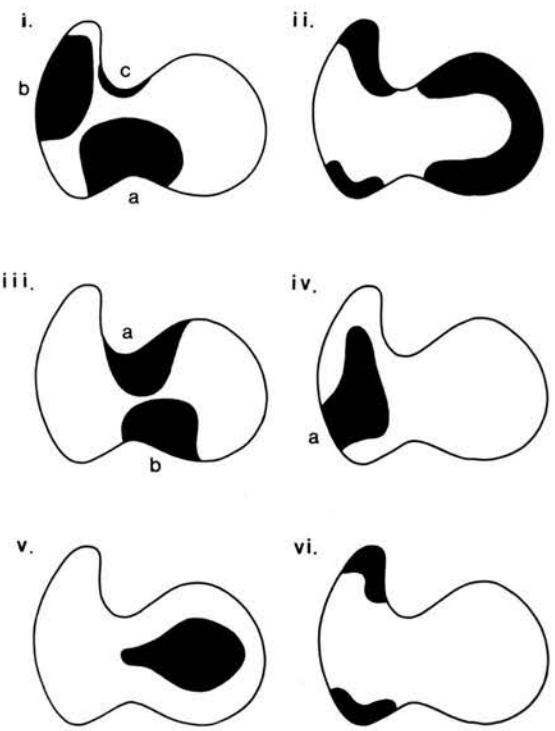


FIG 3: Dorsal view of the growth plate of the proximal end of the femur. The shaded regions are supplied by a specific group of epiphyseal vascular canals (EVCs). 3i: (a) EVCs from caudal perichondrial vessels; (b) EVCs from lateral perichondrial vessels; (c) EVCs from cranial perichondrial vessels. 3ii: EVCs from intracapsular retinacular vessels. 3iii: (a) EVCs from a large cranial retinacular vessel; (b) EVCs from a vessel which branches from a large vessel to the caudal perichondrial vessels. 3iv: EVCs from extracapsular retinacular vessels on the lateral aspect of the trochanter. 3v: EVCs from the teres ligament vessels. 3vi: EVCs from the vessels of the joint capsule and associated connective tissue

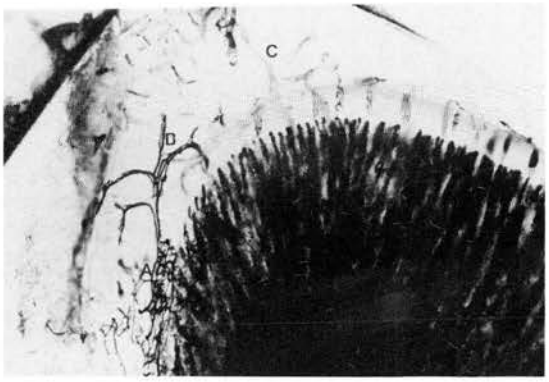


FIG 4: Slab from the right trochanter of a 28-day-old bird. The lateral perichondrial vessels (A) supply an EVC (B) which extends into the trochanteric cartilage. The EVC (C) is from the lateral trochanteric retinacular vessels and supplies the PEVs on the zenith (apex) of the trochanter. ×11

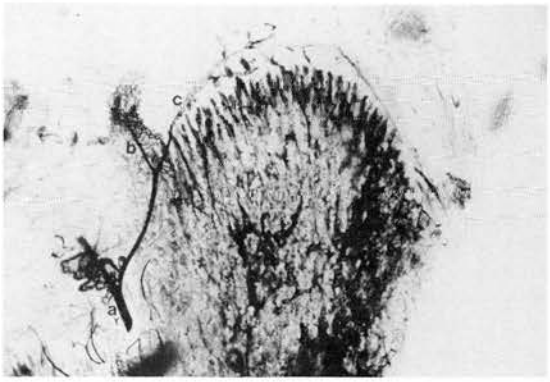


FIG 6: Slab of the caudal aspect of the right proximal femur of a 14-day-old bird. The large vessel (a) branches to supply the perichondrial ring vessels (b) and an EVC to the caudolateral capitus (c). ×11

The caudolateral capitus was supplied by an EVC which was a branch of the supply to the caudal perichondrial ring vessels (Fig 6). This EVC branched to supply the area (b) in Fig 3iii.

The lateral trochanteric surface was covered in a fine reticular network of vessels. From the middle and caudal region of the lateral trochanter these vessels formed EVCs to the area surrounding the zenith (apex) of the trochanter (Fig 3iv).

The teres ligament was the source of the EVCs to the centre of the capitus. Approximately six EVCs radiated from the fovea to form PEVs (Fig 3v and Fig 7).

Small EVCs entered the epiphyseal cartilage of the cranial and caudal trochanteric margins. These EVCs were originating from vessels in the joint capsule and the associated connective tissue (Fig 3vi).

Changes with age

Day-old. The diaphysis and metaphysis in day-old birds contains a cartilaginous core. Growth plate PEVs penetrated the core to connect distally with large medullary vascular channels (Fig 8). These PEVs originated from the caudal perichondrial ring EVCs. Some specimens had poorly developed EVCs from the teres ligament. In these birds the retinacular EVCs extended to supply a greater area of the capitus. Vessels from the retinaculum of the lateral surface of the trochanter formed approximately three EVCs. This was the vascular supply to PEVs of the caudal half of the trochanter, the cranial half being provided with PEVs originating from the lateral trochanteric perichondrial ring vessels. The vessels originating from

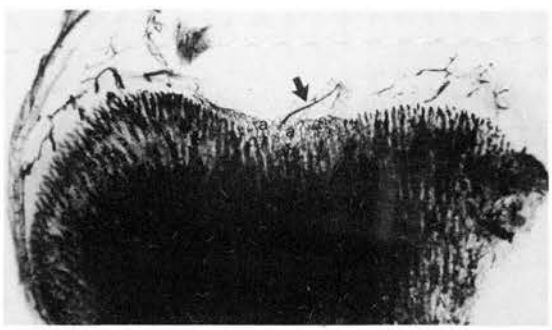


FIG 5: Slab from the left proximal femur of a 42-day-old bird. The plexus of cranial perichondrial vessels (a) do not form EVCs. The large retinacular vessel (arrowed) is on the intra-articular surface of the cartilage. This vessel forms EVCs supplying the craniolateral capitus. ×7

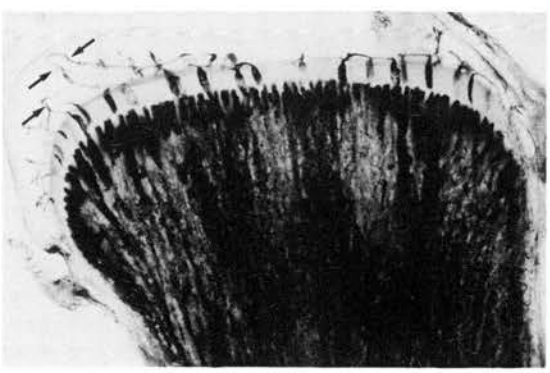


FIG 7: Slab from the right proximal femur of a 14-day-old bird. Three EVCs (arrowed) originating from the vessels of the teres ligament extend through the epiphyseal cartilage to form PEVs. ×11

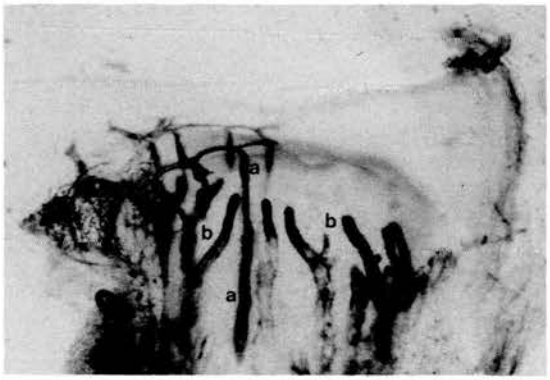


FIG 8: Slab from the right proximal femur of a day-old bird. PEVs (a) originating from the caudal perichondrial vessels, extend into the cartilaginous core. Metaphyseal vessels (b) branch around the periphery of the presumptive growth plate. The epiphyseal cartilage is mainly avascular. ×20

the joint capsule of the cranial and caudal margins of the trochanter formed short EVCs but no PEVs. Branching metaphyseal vessels were present around the periphery of the growth plate.

Day 2. The metaphyseal vessels had arborised to form a completed array below the growth plate cartilage. The cartilaginous core was now smaller and confined in the diaphysis. From the diaphysis, wide medullary vascular channels invaded proximally along the path of the narrower canals formed previously by the PEVs through the physis (growth plate). PEVs no longer penetrated the metaphysis. The EVCs from the anterior trochanteric joint capsule and associated tissue now formed PEVs. All the specimens examined had a well developed supply of EVCs from the teres ligament. The PEVs tended to be arranged in a more regular pattern across the growth plate.

Day 7. The cartilaginous diaphyseal core was no longer apparent. The caudolateral capitus was now supplied by a vessel which branched from the supply to the caudal perichondrial ring. This vessel frequently trifurcated in the epiphysis with branches extending medially, cranially and in a craniolateral direction. The EVCs from the extracapsular retinacular vessels of the lateral aspect of the trochanter (about five in number) extended cranially to supply the cranial margin of the trochanter with PEVs.

Day 28. EVCs from the caudal perichondrial ring extended laterally to supply the caudal trochanter. Lateral trochanteric retinacular EVCs supplied PEVs to the anterior and mid-trochanteric zenith. The cranial margin of the trochanter was supplied by cranial trochanteric EVCs, which originated around the cranial joint capsule. The principal vessels supplying the retinacular tissue and perichondrial ring were found on the cranial and caudal aspects of the femur where the perichondrial and retinacular tissues appeared to be more vascular.

Day 70. The growth plates of the female birds now had shorter PEVs and a reduction in the apparent number of PEVs and EVCs. These changes were not apparent in the male specimens.

Day 105. There was a great reduction in the number of EVCs. In some specimens there were no EVCs derived from the teres ligament, but some EVCs entered the capital epiphyseal cartilage from the peripheral perichondrial and retinacular vessels. The caudal half of the trochanter was fully ossified and was covered in a thin layer of cartilage. The cranial half was mainly avascular hyaline cartilage. There was no evidence of the growth plate cartilage. The metaphyseal vessels formed branching tufts which were invading the cartilage of the capitus and cranial part of the trochanter. By 140 days the epiphyseal cartilage was almost completely avascular and the metaphyseal vessels formed an irregular border between the metaphysis and epiphysis (Fig 9).

Discussion

The developmental pattern of the proximal end of the femur in the fowl can be divided into three stages: formation of growth plate; growth by endochondrial ossification; and cessation of growth.

The first stage is concerned with the development of the growth plate. The erosion of the avascular cartilaginous cores by medullary invasion was first described by Fell (1925) and subsequently by

FIG 9: Slab from the femoral head on 20-week-old fowl. A few EVC remnants (A) are present. There are no PEVs. The metaphyseal vessels (M) form an irregular margin as they invade the hyaline epiphyseal cartilage. ×7

Wolbach and Hegsted (1952). Transphyseal vascular canals penetrating the cartilage core before medullary invasion were not recorded by either author. The penetration of the cartilaginous core of the proximal tibia by descending epiphyseal vessels was demonstrated by Wise and Jennings (1973) in the turkey. In young foals Firth and Poulos (1982) described transphyseal canals and ascribed to these canals a temporary nutritive function, due to the slow development of the metaphyseal vessels. In neonatal pigs (Kincaid and Lidvall 1982) canals are described communicating from the epiphysis to the metaphysis. In the bird the presence of transphyseal vessels penetrating the otherwise avascular cartilage core appears to have a nutritive or maturing role before medullary erosion of the cartilaginous core. The stimulus for penetration of the cartilage core by epiphyseal vessels may be 'blocked' by day 2, due to the presence of a full array of metaphyseal vessels below the growth plate.

The pattern of vessels in the proximal femoral epiphysis is similar but more complex than that of the bird's ulna described by Beaumont (1967). He found a system of vessels originating from the cartilage periphery. The greater complexity of the vascular supply to the proximal femur is due to the vessels from the teres ligament and the retinacular tissue. The reduction in EVCs and PEVs towards the end of the growth period is a similar finding to that in the ulna by Beaumont (1967). These changes occurred at a younger age in the female and probably indicate a hormonal effect causing more rapid and earlier skeletal maturity.

Duff (1985) reported defective trochanteric ossification resulting from epiphyseal hyaline cartilage retention. The regional variation found in metaphyseal vessel invasion of epiphyseal hyaline cartilage would explain the localisation of these lesions. The caudal trochanteric hyaline cartilage was ossified while it was still highly vascular. The cranial cartilage became avascular and was invaded more slowly by metaphyseal vessels at a later stage.

The absence of anastomosis between the EVCs suggests that the result of vascular occlusion would be an area of ischaemia. The vascular supply to some EVC systems is more likely to be susceptible to damage. Such vessels include those of the teres ligament and medial part of the joint capsule. These vessels would be vulnerable to the effects of synovial effusion and joint trauma.

There are minor variations in the area supplied by each EVC system. It would appear that when one system is relatively underdeveloped, neighbouring EVCs can compensate by becoming more extensive.

References

BEAUMONT, G. D. (1967) *Journal of Anatomy* **101**, 543–554

CATTO, M. (1965) *Journal of Bone and Joint Surgery* **47B**, 777–791

DUFF, S. R. I. (1984a) *Research in Veterinary Science* **37**, 293–302

DUFF, S. R. I. (1984b) *Research in Veterinary Science* **37**, 310–319

DUFF, S. R. I. (1985) *Journal of Comparative Pathology* **95**, 384–392

FELL, H. B. (1925) *Journal of Morphology and Physiology* **40**, 417–459

FIRTH, E. C. & POULOS, P. W. (1982) *Research in Veterinary Science* **33**, 159–166

HULTH, A. (1958) *Acta Orthopaedica Scandinavica* **27**, 192–209

KADERLY, R. E., ANDERSON, B. G. & ANDERSON, W. D. (1983) *American Journal of Veterinary Research* **44**, 1805–1812

KINCAID, S. A. & LIDVALL, E. R. (1982) *American Journal of Veterinary Research* **43**, 938–944

LEVENE, C. (1964) *Journal of Anatomy (London)* **98**, 515–538

THERON, J. (1977) *Radiology* **124**, 649–657

THORP, B. H., LYNCH, M. & DUFF, S. R. I. (1986) *Research in Veterinary Science* **40**, 236–240

TRUETA, J. (1957) *Journal of Bone and Joint Surgery* **39**, 358–394

WOLBACH, S. B. & HEGSTED, D. M. (1952) *American Medical Association Archives of Pathology* **54**, 1–12

WISE, D. R. & JENNINGS, A. R. (1973) *Research in Veterinary Science* **14**, 161–166

Accepted July 3, 1985

APPENDIX 5

The same starter and grower diets were used in all the experiments. Each diets contained a vitamin and mineral supplement, incorporated at 2.5Kg/tonne.

Mineral Supplement.

Cu (as cupric sulphate)		70 g
I (as potassium iodate)		8 g
Fe (as ferrous sulphate)		1600 g
Mg (as carbonate)		6000 g
Mn (as carbonate)		2000 g
Zn (as oxide)		1000 g
Ground maize	to	50 Kg

Vitamin Supplement.

Vitamin A		40 million i.u.
Vitamin D3		12 million i.u.
Vitamin E		500 g
Menaphtone		26 g
Riboflavin		80 g
Nicotinic acid		560 g
Pantothenic acid		200 g
d-Biotin		1 g
Ground maize	to	50 Kg

Dietary Analysis.

	Starter	Grower
Protein (%)	22.1	19.0
ME (Kcal/Kg)	3000	3168
Calcium (%)	1.20	1.21
Phosphate (%)	0.50	0.60

LIST OF ABBREVIATIONS

Ac = accessory ossification centre
CA = caudal
CR = cranial
D = dyschondroplastic defect
dis = distal
E = epiphysis
ECRV = extra-capsular retinacular vessel
EOC = epiphyseal ossification centre
EVC = epiphyseal vascular canal
Fib = fibula
FH = femoral head
FT = femoral trochanter
GP = growth plate / physis
Hy = hypotarsus
ICRV = intra-capsular retinacular vessel
IAV = intra-articular vessel
JC = joint capsule
L = lateral
M = medial
MGT = Mason Goldner trichrome
Met = metaphysis
MV = metaphyseal vessel
P = physis
PEV = penetrating epiphyseal vessel
PR = perichondrial ring
prox = proximal
Tib = tibiotarsus
TLV = teres ligament vessel
TM = tarsometatarsus
TT = tibiotarsus
UWB = unilateral weight bearing
VF = vascular foramen
II, III and IV = 2nd, 3rd and 4th metatarsals
3rd = third EOC in distal tibiotarsus